ANNUAL REVIEW OF PHYSIOLOGY

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ANNUAL REVIEW OF PHYSIOLOGY

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VOLUME V

1943

PUBLISHED BY THE
AMERICAN PHYSIOLOGICAL SOCIETY
AND ANNUAL REVIEWS, INC.

ON SALE BY
ANNUAL REVIEWS, INC.
STANFORD UNIVERSITY P.O., CALIFORNIA

ANNUAL REVIEWS, INC.
STANFORD UNIVERSITY P.O., CALIFORNIA

Foreign Agencies

London:

H. K. LEWIS & COMPANY, LIMITED
136 GOWER STREET, LONDON, W.C. 1

Moscow:

MEZHDUNARODNAYA KNIGA
KUZNETSKY MOST, 18

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA BY
GEORGE BANTA PUBLISHING COMPANY

PREFACE

In introducing Volume V of the *Review* there is little to be said that has not already been expressed by your Editorial Committee in the prefaces to preceding volumes. Something, however, can be added by way of emphasis. It is the sense of gratitude that we have endeavored to convey in previous years to the authors of the various reviews,—an intangible thing that grows steadily from year to year. As the war continues to impinge upon every aspect of life with increasing severity and the time available for all but the most essential tasks seems steadily to diminish, the burden of preparing reviews assumes forbidding proportions. Restrictions upon the publication of much important scientific work leave only a residuum but, rather significantly, a very voluminous residuum, of literature in the hands of the reviewer. The unavailability of many scientific journals, referred to a year ago, has continued through 1942 and is almost certain to persist for the duration of the war. What a flood of discovery will be revealed in the first few years of the post-war world when the present barriers to intellectual cooperation melt away!

In expressing our most sincere thanks to all who have contributed to the present volume we do not wish to overlook those who had planned to join us in authorship but were prevented by unanticipated events in getting their manuscripts to press; we refer specifically to the reviews announced in our fall prospectus: "Somatic Functions of the Nervous System" by Philip Bard and Clinton N. Woolsey, "Kidney" by Robert W. Clarke, and "Peripheral Circulation" by Göran Liljestrand and A. Aperia. The last mentioned was sent from Sweden in October but failed to arrive.

Our thanks are also extended to those who have assisted in the routine editorial work and to the George Banta Publishing Company for their splendid cooperation.

J.M.L. M.H.J.
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ERRATA

Volume IV, page 162, line 7 from bottom: *for* 0.002 per cent, *read* 0.002 *M*.

line 8 from bottom: *for* 0.001 per cent, *read* 0.001 *M*.

page 170, line 9: *for* Hoagness, *read* Hoggness.

page 291, line 32: *for Am. J. Clin. Path.*, **10**, 108–13 (1940), *read Am. J. Clin. Path.*, Tech. Suppl. **4**, 108–13 (1940).

page 327, line 29: *for* Scroeder, H. A., *read* Schroeder, H. A.

page 541, line 11 from bottom: *for* (445), *read* (455).

PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF ULTRAVIOLET RADIATION

By Harold F. Blum

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This review is restricted to a discussion of the physiological and pathological effects of radiation comprising a narrow spectral region, approximately wave lengths 2000 Å to 4000 Å. Such a limitation is not to be justified upon photochemical grounds but because certain photobiological effects produced by such radiation are closely related. This is due to the fact that they involve substances, common to all living systems, which absorb specifically in this region of the spectrum. Only the fundamental character of these processes and their physiological and pathological aspects are discussed, but even within this field further restriction has been made. Systemic effects, which result from the superficial action of ultraviolet radiation and which are not as a rule produced specifically by this agent (72), have been largely neglected. Likewise, therapeutic and sanitary uses of ultraviolet radiation are not touched upon, nor are photochemical studies on biological materials except in a few instances.

Fundamental Processes

It is now generally believed that ultraviolet radiation of wave lengths shorter than about 3200 Å affects cells by direct action on cell constituents which absorb these specific wave lengths, and that the type of absorbing substance so affected is either protein or nucleic acid.[1] The results of such action are generally lethal or injurious but may occasionally manifest themselves as responses suggesting stimulation.

The light absorber.—It is a current question whether proteins or nucleic acids, both of which are photolabile (63), are the light absorbers for such processes. The answer has been sought by comparison of action spectra[2] with the typical absorption spectra for

[1] These wave lengths are effectively removed by ordinary window glass. Hence photobiological effects which are prevented by the introduction of a screen of this material usually belong to this group of phenomena.

[2] This term is applied to the relationship between wave length and effective-

the two types of compound, both of which absorb wave lengths longer than 3200 Å to only a slight extent. Proteins show a maximum of absorption at about 2800 Å, and nucleic acids at about 2600 Å. In a number of instances the maximum of the action spectrum is close to one or the other value, and its shape agrees reasonably well with that of the corresponding absorption spectrum (39, 62, 64, 70).

It seems probable that proteins are the light absorbers in certain processes, nucleic acids in others; and this suggests that the primary reaction occurs sometimes in the cytoplasm, sometimes in the nucleus. For example Giese has compared the effects of wave length 2804 Å, which approximates maximum protein absorption, with that of wave length 2654 Å, which approximates maximum nucleic acid absorption. Cell division is more effectively retarded in paramecia (40), in the fertilized eggs of the sea urchin (39), and the worm *Urechis* (42) by wave length 2804 Å than by wave length 2654 Å; these facts suggest that protein is the light absorber and consequently that the effect is localized principally in the cytoplasm which is the structure reached first by the radiation. Exposure of sea urchin sperm to such radiation before fertilization of the egg also retards cleavage, and in this case wave length 2654 Å is more effective than wave length 2804 Å; this indicates nucleic acid is the light absorber (41). The sperm head is almost entirely nucleus and is reached by most of the radiation.

These observations suggest that either protein or nucleic acid, and either nucleus or cytoplasm may be affected, depending on the nature of the system and the experimental conditions. Unfortunately, the general similarity of these absorption spectra, and limitations imposed on the measurement of action spectra by the nature of the systems studied, e.g., screening action of the superficial layers, render comparisons uncertain. Hence, conclusions drawn from this type of measurement must always be tempered with caution.

The absorption spectra of proteins and nucleic acids do not end at 3200 Å although they are relatively slight beyond that wave length. High intensities of radiation of longer wave length ultraviolet radiation, e.g., the 3650 Å line of the mercury arc, pro-

ness in bringing about a given photobiologic effect. Under ideal conditions the action spectrum and the absorption spectrum of the substance acting as the light absorber should agree (7).

duce effects (62, 82) which may represent primary action on these substances or, in some instances at least, result from absorption by another component of the system (57).

Susceptibility.—Rentschler, Nagy & Mourmonseff (89) find that if intensities are so adjusted that equal energies are applied over periods of time ranging from a few microseconds to several minutes, the killing effect on *E. coli* is the same. Thus, the reciprocity law holds for a wide range of intensities. However, when the intensities are so adjusted that the range of exposure time extends over many hours, this simple relationship breaks down, and it is found that higher intensities applied for short periods are less effective than low intensities for long periods. The lower effectiveness at short periods is attributed to differences in susceptibility of the bacteria at different phases of their life cycle, since the chance of encountering the organisms in a susceptible phase increases with length of exposure. The effectiveness falls off again with very long low intensity exposures due to the increase in number of cells per colony. Raising the temperature results in increased killing at shorter periods, presumably by speeding up the life cycle since the energy required to kill is independent of temperature at very short exposure periods. Other evidence of influence of the life cycle on lethal effectiveness is presented by these investigators. It is deduced that the hypothesis that a specific sensitive spot must be hit in order to kill is untenable, unless it is arbitrarily assumed that the size of the spot changes during the life cycle.

Susceptibility of *Fucus* eggs increases progressively following fertilization (88, 100). Brandes (16) finds that the resistance of the cells of frog embryos to ultraviolet radiation increases with the age of the embryos.

Mutations.—Ultraviolet radiation may induce relatively high incidence of mutations (e.g., 81). Stadler (94) points out that in maize pollen ultraviolet radiation induces a much higher proportion of mutations than do x-rays and that the former characteristically induces gene mutations but few chromosome aberrations, whereas the opposite is true for the latter (see 97).

Determination of action spectra for the induction of mutations by ultraviolet radiation in fungi (61, 62), liverwort spores (68), and maize pollen (95) all agree in placing the maximum near 2600 Å, these findings indicating nucleic acid as the light absorber.

Adequate studies to determine this point have not been carried out on animal forms. Studies on the sperm of *Drosophila* have been made (25, 81), but unfortunately the sperm must be irradiated through the body wall, and selective absorption by that structure hinders determination of the true action spectrum (28, 62). Since the evidence points to nucleic acid as the light absorber, photochemical action may be localized in the chromosomes in organisms possessing these structures, and possibly the gene is affected directly by the radiation (62).

Cell division, growth, etc.—Giese (39 to 42, 46) finds that ultraviolet radiation retards cell division of numerous species of protozoa and eggs but has not observed acceleration of this process. Loofbourow and others (22, 23, 24, 75 to 79), on the other hand, find that increase in growth, and presumably cell division rate, follows exposure of bacteria and yeast cultures to sublethal ultraviolet radiation. This effect is not specific but due to growth promoting factors liberated by cells injured by other agents as well as by ultraviolet radiation (75). The growth promoting factors seem related to nucleic acids. They are liberated in greater amount by living than by dead cells (75). Toxic factors are also produced by such treatment (78).

Chase (20) reports increased growth of algal cultures after exposure to ultraviolet radiation, but the changes persist for months and may be mutations.

Delay in germination and growth may occur in fungous spores which survive exposure to ultraviolet radiation (63, 103). Retardation of growth persists for varying lengths of time, but this is not correlated with mutation. Dimond & Duggar (26) observed a large proportion of abnormalities in germination. They eliminated by experiment the possibility that these resulted from formation of toxic products or increase in space provided by death of part of the spores. They did not find stimulation of germination at any dosage of radiation. Ultraviolet radiation applied to protozoan cysts causes a delay in excystment (44).

Respiration.—Cell respiration is usually depressed by ultraviolet radiation. However, stimulation is sometimes observed, and Giese (45) has found that this effect may be obtained consistently with cultures of yeast in which respiration is at a low level.

Action on protein systems.—Enzymes, viruses, and other essentially protein systems are altered by ultraviolet radiation.

Physical changes in proteins are detected earlier than chemical, and in some systems are most conveniently measured by inactivation of a specific property, e.g., enzymatic activity. "Quantum yields" have been determined or estimated for inactivation of a number of enzymes. The molecules must receive a very large number of quanta before being inactivated. Values arrived at range from 10^2 to 10^5 quanta per molecule (71, 98, 99).

Ultraviolet radiation may become a useful tool in immunology, since it is possible to inactivate viruses without complete loss of immunizing potency, the latter being retained to a considerable extent in some instances (18, 60, 85, 92).

Effects on Specialized Cells

Harvey (53) has studied the effects of short flashes of intense ultraviolet radiation on a variety of cells. In general the effects are comparable with those produced by longer less intense exposures. Ameboid and ciliary movement are inhibited as a rule, while the myonemes of some protozoa are caused to contract. Protoplasmic streaming is stopped in *Nitella* but continues after a short time; this effect is comparable with that of various stimulating agents. Striated muscle fibers (54) respond with contracture, but the normal twitch response is not elicited. All these responses seem explainable in terms of injurious effects resulting from sudden photochemical alteration of protein components of the cells.

Whitaker (88, 100 to 102) has described unilateral rhizoid formation and plasmolysis in the eggs of *Fucus* exposed to ultraviolet radiation from one side only. Very interesting opposing effects are brought about by visible radiation, presumably acting through the photosynthetic mechanism (102).

Studies on a luminous bacterium by Giese (43) show that respiration, reproduction, and luminescence are all depressed by the action of ultraviolet radiation. Depression of respiration possibly results from direct destruction of the respiratory enzymes by the radiation. *Cypridina* luciferin and luciferase are inactivated by ultraviolet radiation (19); this change probably accounts for the reduction in luminescence.

Effects on the Skin

Erythema of sunburn.—Two recent attempts to analyze the action spectrum for the erythema of sunburn in man arrive at

different conclusions. Mitchell (84) selects protein as the light absorber, whereas Hamperl, Henschke & Schultze (51) select nucleic acid. Both take into consideration the screening effect of the corneum, which acts as a nonparticipating layer having selective absorption and scattering properties. It must be difficult to discover by such a method of analysis which type of compound is the major light absorber for the primary photochemical reaction because of unavoidable errors of measurement in such a system. The histological evidence that the nucleus is primarily affected, offered by Hamperl, Henschke & Schultze (51), is open to criticism. They credit the degeneration of the most superficial nuclei of the epidermis after exposure of the skin to ultraviolet radiation to direct action on these nuclei which must receive more radiation than those more deeply placed. However, the degeneration of the nuclei may be merely an accompaniment of cornification of the superficial cells of a hyperplastic epidermis.

Whatever analysis of the action spectrum is accepted, it is obvious that the primary changes are similar to those produced in cells in general by wave lengths shorter than 3200 Å (see p. 1). Miescher (82) has described production of erythema by high intensities of longer wave lengths characterized by immediate appearance, as contrasted with that produced by shorter wave lengths which has a considerable latent period. A different mechanism may be involved, but it seems likely that this effect also results from action on proteins or nucleic acids (see p. 1). As Miescher suggests, the absence of a latent period may indicate direct action on the blood vessels, which are reached by these longer wave lengths but not appreciably by those shorter than 3200 Å. This phenomenon might explain the pronounced effect on blood pressure caused by the carbon arc (74), as contrasted with the mercury arc, since the emission of the former includes the strong cyanamide bands in this region.

The erythema of sunburn is generally credited to the action of a histamine-like dilator substance released by injured epidermal cells, which diffuses to the underlying minute vessels. A resume of the evidence (7) leads to the conclusion that this substance is elaborated or released by the cells as a result of injury, rather than formed directly by specific photochemical action. Histamine-like substance has been demonstrated by Laurens & Kolnitz (74) in the blood of hypertensive dogs after exposure to carbon arc radia-

tion. In accord with this general picture, Laurens & Graham (73) find that desoxycorticosterone opposes the reduction of blood pressure brought about by carbon arc radiation in either normal or adrenalectomized dogs.

Pigmentation.—Henschke & Schultze (57) provide an answer to the vexed question of pigment production by long wave lengths of ultraviolet radiation. They confirm, in general, the findings of I. Hausser (55) but point out errors in her action spectrum analysis and conclusions. Pigmentation resulting from exposure to sunlight has two more or less independent phases, each with a different action spectrum: (*a*) formation of new pigment has the same action spectrum as the erythema of sunburn and probably is a sequel to that process; and (*b*) darkening of pigment already present in a bleached form upon exposure to wave lengths between 3000 Å and 4200 Å, the action spectrum having a rather flat maximum at about 3400 Å.

Besides differing in action spectrum, the darkening process requires very much greater amounts of radiation than the formation of new pigment and begins almost immediately upon exposure instead of after a latent period of a few days. Moreover, the former depends on the presence of oxygen whereas the latter does not. Miescher & Minder (83) believe that this darkening represents the oxidation of melanin which is present in a reduced leucoform. They find the darkening of pigment in living skin brought about by ultraviolet radiation to be comparable with that brought about in dead skin by ultraviolet radiation or by heat. Hamperl, Henschke & Schultze (52) find that the pigment is differently distributed in the epidermis in the two phases of tanning.

Miescher & Minder's hypothesis is supported by Figge's demonstration (36) that melanin behaves as an oxidation-reduction indicator, the oxidized form being dark, the reduced form bleached. The possibility that melanoid pigment (29) may be a reduced form of melanin should be explored, since this would fit with a number of the recent findings herein described. The darkening of the skin of previously sunburned areas which results from the administration of sex hormones (31, 50) and from other agents (65) may be due, in part at least, to oxidation of reduced melanin.

Henschke & Schultze (58) point out that the greater degree of tan observed after exposure to sunlight as contrasted with that following exposure to the mercury arc can be accounted for on the

basis of the greater intensity in sunlight of wave lengths longer than 3200 Å which bring about the darkening of pigment already in the skin. Their findings explain the tanning curve presented by Luckiesh & Taylor (80).

The color of skin is, of course, dependent upon more than one pigment. From analysis of measurements of light reflected from the skin, Edwards & Duntley (30) attribute the characteristic brown color following sunburn principally to melanin, although reduced hemoglobin may be important in early stages.

Schemes which postulate formation of melanin in the skin by the direct action of ultraviolet radiation on some part of the β-3,4-dihydroxyphenylalanine (dopa) system have been formulated (e.g., 90). The picture presented above indicates that new pigment formation results from cell injury rather than from direct action of ultraviolet radiation, and there are other arguments which favor the former conclusion (7).

Protection against sunburn.—It is not generally recognized that thickening of the corneum with resulting increase in its screening action, rather than melanin formation, is the principal factor in the increased resistance to sunburn which follows exposure to sunlight. Additional evidence for this is provided by the demonstration that transmission of ultraviolet radiation by the epidermis of the albino mouse is greatly reduced by repeated exposure of the animals to this agent (13, 67). The albino mouse develops no melanin, but thickening of the epidermis does result from such exposure.

The effectiveness of artificial protection against sunburn must depend upon absorption of the effective wave lengths before they reach the epidermal cells. This is recognized in the studies of Bachem & Fantus (3), Kimmig & Düker (66), and Henschke (56), who considered the absorption spectra of such preparations and their ingredients. On this basis many of the preparations designed for this purpose appear almost worthless. Strakosch (96) has demonstrated the importance of the ointment base in which the light absorbing material is carried; this may be due to alteration of the scattering properties of the corneum as well as to scattering properties of the base itself.

Antirachitic action.—Formation of vitamin D bears no relationship to the effects described above for wave lengths shorter than 3200 Å but, for reasons which appear fortuitous from a biological

standpoint, occurs in a spectral range having approximately the same long wave length limit. Measurements of the action spectrum agree quite well with the absorption spectrum of provitamin D. Bunker, Harris & Mosher (17) find a close parallel between quantum yield and the action spectrum, which would suggest that all the incident energy is utilized in activation of the provitamin. The action spectrum obtained by Knudson & Benford (69) is in better agreement with the absorption spectrum of the provitamin, and would suggest that only a thin superficial layer is being activated. Whatever the cause of the discrepancy in the action spectra, both observations emphasize that the formation of the vitamin occurs near the surface of the skin, since if the photochemical reaction occurred at any significant depth it would be subjected to modification from screening by the more superficial layers as occurs in the erythema of sunburn.

Rapoport & Rubin (87) find that administration of vitamin D or exposure to sunlight enhances the weight loss of rats receiving lead in the diet, presumably by increasing the assimilation of the lead. They suggest that greater incidence of lead encephalopathy in infants during summer months may result from similar action of sunlight.

Abnormal sensitivity.—Certain photosensitivities of man represent abnormalities of the normal response of skin to ultraviolet radiation. These have been reviewed up to a recent date by the writer (7). Sonck (93) has since reported photosensitivity associated with lymphogranuloma inguinale (venereal lymphogranuloma). Longer wave lengths of the ultraviolet are most effective, and high intensities must be required since sunlight was effective in eliciting the abnormal response whereas the mercury arc was not. This suggests that these wave lengths are more effective because of their deeper penetration (see p. 6) or that another response is involved than that of normal sunburn. The maximum absorption of porphyrins lies in this region of the spectrum, but the absence of sensitivity to the visible spectrum argues against photosensitization by these compounds. The patients showed very slight if any porphyria. Pending further investigation it seems probable that this photosensitivity is an aberration of the normal skin response and that it has an allergic basis. Its possible relationship to polymorphic light eruption is of interest if only because

confusion in diagnosis might occur. Abramson (1) has described a case of urticaria caused by wave lengths of the same approximate region.

Callaway (21) has demonstrated passive transfer of photosensitivity, apparently to wave lengths shorter than 3200 Å, by injection of the blood serum of the patient into the skin of a normal individual. Scattered earlier observations of the same type have been made (7).

If sulfanilamide is injected intradermally in high concentration, it exacerbates the normal sunburn response of skin in the neighborhood of the injection (8, 9, 34, 35). An inflammatory response, probably allergic, develops in some individuals if the photosensitivity is repeatedly evoked. This response may be related to the photosensitivity which sometimes follows sulfonamide therapy.

Cancer.—Evidence converges from a number of sources to indict the ultraviolet radiation of sunlight as a major factor in human cutaneous cancer (6). For this reason, and because it provides a useful method for studying the mechanism of carcinogenesis, the induction of tumors by ultraviolet radiation has received considerable attention.

The long wave length limit for experimental induction of tumors is approximately 3200 Å (37, 91). The effectiveness of wave lengths shorter than this limit is determined largely by the screening action of the corneum (15). Thus the primary action of the radiation is probably on protein or nucleic acid of the cells.

The tumors induced in the ears of albino mice by ultraviolet radiation (48) are predominantly sarcomas arising from connective tissue, with a smaller proportion of carcinomas arising from epidermal tissue. In contrast, malignant tumors of the skin of man virtually all arise from the epidermis. The discrepancy is accounted for by differences in depth of penetration of the radiation into the two types of skin (67). In man little or no radiation of wave lengths shorter than 3200 Å penetrates below the epidermis, whereas in the albino mouse a great deal reaches the sub-epidermal tissues. Heuper's (59) observation that hairless rats exposed to ultraviolet radiation develop a tremendously thickened corneum but few tumors is further evidence of the importance of penetration of the radiation and of the protective action of the horny layer (see p. 8).

The interval between exposures to ultraviolet radiation is an

important factor in determining the rate of induction and the relative incidence of tumor types. The induction time is shorter, and the ratio of carcinomas to sarcomas greater the more frequent the exposures. For a given frequency of exposure the tumor time varies, within limits, inversely with the dose, but above a certain value the induction time does not decrease with increase in dose. The carcinoma-sarcoma ratio does not vary with dose *per se* (10, 11, 49).

The hypothesis that sterols in the skin are changed by ultraviolet radiation into carcinogenic compounds receives no support from recent experiments. Bergmann and co-workers (5) have been unable to produce carcinogenic substances by exposure of various sterols to ultraviolet radiation *in vitro*. In accord with earlier findings, Baumann & Rusch (4) found no increase in tumor induction by ultraviolet radiation in mice fed a high cholesterol diet. Evidence from wave length studies (15) indicates that the cells are directly affected by the radiation and that the corneum, which must contain a large proportion of the sterols of the skin, does not participate in carcinogenic action.

Some of the polycyclic hydrocarbons are both carcinogens and photosensitizers for photodynamic action, but a direct relationship between the two mechanisms is highly improbable (7). Doniach & Mottram (27) and Morton, Luce-Clausen & Mahoney (86) find that light opposes the induction of tumors of the skin by painting with such hydrocarbons. It is unnecessary to postulate a direct relationship between carcinogenesis and photodynamic action to explain this finding. Both processes must be limited by the amount of the carcinogen taken up by the cell (7, 47). Those cells that take up most carcinogen are the most likely to become cancer cells, but these same cells having taken up most photosensitizer are most susceptible to injury by photodynamic action if reached by light. Thus in animals exposed to light a certain number of potential cancer cells would be destroyed by photodynamic action, and tumor induction correspondingly delayed.

The possibility that the carcinogenic action of ultraviolet radiation resides in its ability to induce mutations in somatic cells has been suggested (e.g., 81). There are many interesting parallels between the two phenomena, and the hypothesis must be seriously considered although the existing evidence may be interpreted in other ways.

Growth and Metabolism

Ultraviolet radiation of wave lengths shorter than 3200 Å slows normal increase and may bring about actual decrease in body weight of mice. No stimulating action occurs at any dosage. The effectiveness of wave lengths within this range is not greatly different if the screening action of the epidermis is taken into account (12). Ellinger (32, 33) attributed this effect to increased activity of the thyroid gland resulting from stimulation by histamine released in the skin as a result of the action of ultraviolet radiation. Blum, Grady & Kirby-Smith (12), on the other hand, account for the reduction in body weight by decreased food intake and find no evidence of qualitative effects on metabolism or general systemic changes. Hypervitaminosis is ruled out as a contributing factor (14).

Effects on the Eye

While scotopic vision of the human eye is generally thought to end at about 3900 Å, shorter wave lengths are perceived although the sensitivity is not great. Recent studies by Goodeve, Lythgoe & Schneider (38) indicate that the mechanism is not essentially different from scotopic vision at longer wave lengths. Divergence between the spectral photosensitivity of visual purple and spectral sensitivity of the retina at these shorter wave lengths is apparently due to absorption by the lens. An aphakic eye showed greater sensitivity in this region. The short wave length limit for the normal eye is approximately 3100 Å, for the aphakic eye 3000 Å.

Wave lengths shorter than 3200 Å produce lesions of the cornea of mice, which include malignant growths (49). Apparent high incidence in the southern states of tumors of the limbus of the eye in man paralleling that of human cutaneous cancer (2) suggests that sunlight may be a factor in the development of such tumors.

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PHYSICAL PROPERTIES OF PROTOPLASM

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Protoplasm and the Cell

The physical basis of life was first identified by Dujardin (1835) as a flesh-like substance which "appears perfectly homogeneous, elastic, contractile and diaphanous, refracting light more than water and less than oil." This original description of some of the physical properties of protoplasm would scarcely be challenged today, but its accuracy was unfortunately marred by the further statement: "One can distinguish in it no trace of organization, neither fiber nor membrane nor any appearance of cellular form."

We can well understand this discrepancy when it is recalled that Dujardin's extraordinary discovery was quite inadvertently made during his controversies with Ehrenberg who maintained that Infusoria were highly complex, complete organisms. Also, the description of sarcode antedated by several years the formulation of the Cell Theory (1838 to 1839).

The discrepancy has for us, however, more than historical interest. It should help us bear in mind during this review that our concepts of protoplasm cannot be divorced from those of the cell. It further suggests the need of briefly clarifying those concepts as an adopted basis for the selection and treatment of the researches herein to be reviewed.

The cell was early recognized as the unit of structure and function for all forms of life. This unit was then identified as a protoplasmic unit, the constituents of which were surely molecular. Assuming the analysis of animate and inanimate nature thus to be comparable, how might their differences be accounted for? Their essential differences would seem to correspond to differences in the degree of their molecular organization (1), bearing in mind, however, that the organization of protoplasm does not stop short of the protoplasmic unit, or cell. This unit, moreover, is never homogeneous but, wherever found, it is typically differentiated into

cytoplasm and nucleus with their respective membranes. Thus, the cell may rightly be defined as a unit of differentiated protoplasm (2).

Cells differ markedly, however, in the degree of their protoplasmic differentiation, depending not only on their level in the phylogenetic scale but also on their stage in ontogenetic development. Each of all forms of life begins its individual existence as a so-called undifferentiated or primordial cell which by protoplasmic differentiation or also by cell division and cellular differentiation becomes the characteristic adult organism. Accordingly, the primordial cell represents the stage of least protoplasmic differentiation so that the physical properties of its protoplasm should find there their simplest, most generalized expression.

Developmental approach.—In this review of current studies on physical properties of protoplasm, covering chiefly the published findings of the past two years, the developmental approach, as indicated above, has been adopted since it shifts the emphasis primarily to stages of least protoplasmic differentiation and thereby offers the prospect of duly limiting the review in scope and content on a topic that obviously has almost limitless ramifications.

Further limitations would seem to be feasible and necessary. In view of this the general plan has been simplified to include only two principal subdivisions. The first deals mainly with descriptive studies of some physical properties of the hyaline cytoplasm (devoid of its inclusions) and its plasma membrane. Properties of the nucleus are ably discussed in another chapter by Dr. Jack Schultz. The second subdivision is concerned with the physical structure of the cytoplasm and of some other physical systems, studies on which have yielded results that apparently help to interpret the physical properties of protoplasm generally. Many valuable papers, accordingly, have been omitted. Effort has been made to find a theme which might not only represent some present research trends but also help tie together the reviewed accounts, even at the risk of some serious omissions. These arbitrary limitations appear all the more allowable because of the publication of a number of relevant symposia, especially those held in commemoration of the pronouncemnt of the cell theory one hundred years ago. Advantage has gratefully been taken of these various publications in preparing this review.

Physical Properties

Identity of protoplasm.—The cell as commonly identified comprises not only protoplasm but also the dispensable products of protoplasm. These include the extraneous coats (3,4) surrounding the plasma membrane, and a diversity of cytoplasmic granules, globules, and other microscopic inclusions. It has been shown by microdissection and with the ultracentrifuge that the cell may be freed of its extraneous coats and its various inclusions, yet continue to live and reproduce (5). Thus the sea urchin egg may be elongated by centrifuging and its visible inclusions greatly condensed at the two poles. The poles with their inclusions can then be dissected off leaving a hyaline, inclusion-free central region that will fertilize and divide. The ameba and the ciliate *Euplotes* can likewise be freed of their inclusions, following which they will reproduce and inclusions will again appear in the daughter cells. Consequently, the hyaline ground substance of the cell which is indispensable to its life and reproduction may properly be identified as protoplasm (6). In this review the term protoplasm will accordingly have reference only to the hyaline ground substance along with its protoplasmic differentiations such as plasma and vacuolar membranes, cilia, fibrils, and framework.

Protoplasmic states.—Among the earlier controversies on properties of protoplasm, perhaps none were more polemic than those having to do with its physical state. Brücke's contention that a fluid protoplasm could not be contractile illustrates one of the issues. Fluidity was, of course, conspicuous in protoplasmic streaming and cyclosis. Satisfactory answers had to await improved techniques, by which it was shown that cells may differ in their solid or fluid consistency and that both protoplasmic states may occur simultaneously in a given cell. Moreover, each physical state is reversible (7 to 12). These concomitant and reversible physical states are obviously physical differentiations of the cell and may be regarded essentially as incipient phases of protoplasmic differentiation.

By methods of micromanipulation (7), of ultracentrifuging (10 13, 14), and application of high hydrostatic pressures (15, 16, 17), it has been shown that the cytoplasm of plant and animal cells and of unicellular organisms commonly comprises a cortical zone or

ectoplasm and an inner zone or endoplasm. The ectoplasmic layer is relatively firm and elastic and the bulkier endoplasm is often distinctly fluid. These regions are never sharply delimited but they are frequently clearly differentiated both in consistency and in the kind and number of their inclusions (12). Monné (6) observed that the ectoplasm of ovocytes, spermatocytes, amebocytes, leucocytes, and fibroblasts of pulmonates is mostly free of inclusions although it is lipoid-rich; it contains the Golgi apparatus, and it weakly exhibits positive birefringence with reference to the cell's radius. The endoplasm is lipoid-poor and contains the mitochondria. The relative consistencies of the ectoplasm and endoplasm were found to vary during meiosis and mitosis.

Differences in protoplasmic consistency are referable not only to its cortical and inner regions (18) and to the phases of cell division (9, 10) but also to the stages of cellular differentiation. Thus the protoplasm of the immature ovum of *Arbacia* apparently shows no regional differences in consistency. Along with the breakdown of the germinal vesicle, however, a medullary region becomes relatively fluid leaving a cortical, more solid, layer some 4 or 5μ in thickness (10, 15). Also the entire cytoplasm of various fully differentiated tissue cells, such as of nerve and muscle, has been shown by microdissection to be fairly solid throughout (7). Pollister (19) observed that the mitochondria in nerve and muscle cells were arranged in line with the fibrillae, presumably corresponding to an orientation of elongated protein molecules of the interfibrillar cytoplasm. In epithelial cells, mitochondria were oriented parallel to the long axis but, in dividing leucocytes, were radially oriented, coinciding with the astral rays.

Siminovitch & Levitt (18) noted in plant cells a gelatinous cortex and fluid endoplasm comparable with those of animal cells. In vacuolate cells having only a thin protoplasmic layer, this relatively solid-fluid differentiation was demonstrable by the Chambers' oil-drop technique. A drop of oil applied by means of a micropipette would snap into the outer surface of the protoplasm and become biconvex. Upon application of pressure and stretching of the protoplasm, the drop became concavo-convex and the endoplasm flowed freely in and out of the angular space between the edge of the drop and the surface boundary of the vacuole.

In highly differentiated protozoa, the cortical and inner re-

gions of the protoplasm may be readily identified. The former is frequently very firm and the latter exceptionally fluid. By means of the centrifuge, Brown (20) appears to have demonstrated that the endoplasm of *Paramecium*, which was formerly reported to be decidedly viscous, is actually quite fluid. The paramecia were fed on "dust iron" and the velocities of movement of the intact food vacuoles were measured at relatively low speeds of the centrifuge. The viscosity of the endoplasm was thus found to have a maximum value of 0.5 c.g.s. units which is several thousand times less than values previously reported.

Reversible gelation.—The fluid state of protoplasm, as early observed in streaming and cyclosis, came to be entirely reconcilable with the firm state of muscle and nerve cells upon recognition of the ready reversibility of these physical states, as in ameboid cells and during cell division. The significance of these reversible changes has been enhanced through identifying them as reversible gelation phenomena essentially comparable with the sol-gel transformations of other physical systems. This is the basis of modern investigations.

A conspicuous example of the reversible gelation of protoplasm is, of course, ameboid movement. A clear description of this has been made by Mast (21) on *Amoeba proteus*. His terminology has now been widely adopted for ameboid cells generally and it might profitably be extended to other cells in studies on gelation changes. The cytoplasm of *A. proteus* thus comprises a thin outer plasmalemma which encloses a relatively solid cortex, the plasmagel. This in turn surrounds the fairly fluid plasmasol. Both the plasmagel and plasmasol contain many and diverse inclusions, but between the plasmagel and plasmalemma occurs a thin fluid hyaline layer which may vary in thickness.

During locomotion, the plasmalemma is attached to the substratum and to the adjoining plasmagel; the plasmagel at the posterior end is transformed into plasmasol, which flows forward to the anterior end and is there transformed into plasmagel. The foreward flow of the plasmasol is due to the contraction of the plasmagel at the posterior end and expansion at the anterior end, owing to differences in its elastic strength in these two regions.

Lewis (22) has described similar gelation changes in white blood cells, macrophages, connective tissue cells, and malignant sarcoma cells during locomotion and cell division. The role of automatic

contractility of the plasmagel was particularly emphasized for locomotion and cell division, comparable with the contraction of a blood plasma clot about a liquefied area. This, accordingly, does not involve a turgid system as conceived by Mast for the ameba. Plasmagel contractility appears to represent a basic mechanism which locomotion and cell division share in common (9). During the division of mouse sarcoma cells, numerous constriction rings appeared and disappeared irregularly before, during, and after cleavage. The rings varied in depth, some nearly pinching the cell in two. Division was thereby eventually accomplished. Similar rings that behaved peristaltically in ameboid cells effected locomotion. These constriction rings represented thickenings of the plasmagel. There is now considerable evidence indicating that a thickening and constriction of the cortex in the division plane of cells generally can account for their cytoplasmic fission (7, 22, 23, 24).

Similar gelation changes apparently obtain also in protoplasmic streaming. Rhythmic pulsations during the locomotion of the slime mold, *Physarum polycephalum*, have been reported (25,26,27) that may be comparable with the peristaltic behavior of the constriction rings in ameboid cells. Seifriz (27) would account for protoplasmic streaming and cyclosis as due to a rhythmic contraction of the gelated layer that surrounds the channel of flow.

Marsland (10) showed by imposing high hydrostatic pressures on *Elodea* that its rate of streaming varied with the pressure applied. Hydrostatic pressure likewise effected gelation changes in ameboid movement and in cell division. With increasing pressure the plasmagel behaved as a fluid cylinder, then a fluid sphere. In dividing marine eggs the furrowing involves an increase in gelation and constriction of the cortex only in the division plane. Hydrostatic pressures applied during cleavage blocked the furrowing process and the cell rounded up. Pease & Marsland's (11) studies of cleavage in *Ascaris* eggs related hydrostatic pressure effects with those produced by centrifuging.

These pressure effects apparently have demonstrated reversible gelation during ameboid movement, protoplasmic streaming, and cell division, showing also that the inhibiting effect on each activity was proportional to the liquefying effects on the gelated protoplasm. Marsland (10) notes a second group of cells, however,

including muscle, nerve, ciliated, and flagellated cells, whose activity is not inhibited but may be augmented by comparable pressures. Also the pressure effects on their activities were not proportional to the effects on their gel-sol transformations.

A generalization was proposed by Heilbrunn (14, 28) that gelation, involving contractility in muscle cells, is normally effected by calcium which is bound in the protoplasmic cortex and upon stimulation is released to the cell interior. Alsup's (29) results are consistent with this view. He showed by centrifuge tests that intense illumination increased the viscosity of the plasmagel of *Amoeba dubia* about 30 per cent but that the effect depended upon the availability of calcium. A similarly induced increase in the viscosity of the plasmasol was preventable by the removal of calcium from the medium.

Chambers (5) records a coagulating effect on the protoplasm of sea urchin eggs that were torn in a calcium chloride solution. In a solution of potassium chloride, however, liquefaction of the exposed protoplasm resulted along with its dispersion in the solution. The fluidity of protoplasm of the intact, fertilized egg was greatly increased when immersed in sea water diluted with distilled water to two thirds of its original concentration. The protoplasm became so fluid that the granular inclusions showed violent, oscillatory movement which was evident even in transmitted illumination. Cleavage continued but at a lower rate.

Northen (30 to 33) obtained a decreased viscosity in the protoplasm of *Spirogyra* subjected to various stimulating and anesthetic agents, to the electric current (2 ma. for 10 min.), to carbon dioxide, temperature changes, mechanical impacts, and centrifugal force. Muir (34) found that a decrease in viscosity, also in *Spirogyra*, was effected by bile salts (sodium taurocholate and sodium glycocholate) and by sodium and magnesium oleates. Kitching & Pirenne (35) confirmed earlier findings that various myxomycetes stop all streaming movements in the absence of oxygen and found that streaming ceased also over an atmosphere of hydrogen, or of oxygen and hydrogen mixtures when the oxygen tension was less than 3 mm. Hg.

Elasticity.—The physical differentiation of protoplasm into an outer gelated zone that intergrades with a plastic, more fluid interior, and the reversibility of these states experimentally and in na-

ture, may accurately describe the grosser, more conspicuous properties that have been amply demonstrated for many cells. An insight into the finer organization of protoplasm, however, may be better gained through its physical property of elasticity. A satisfactory demonstration of this property has been difficult. Seifriz (36) who has long emphasized the significance of the elasticity of protoplasm points out that it may be readily demonstrated in thicker protoplasm and even in thin protoplasm by pulling out a strand by microneedles. Upon release, the strand usually recoils rapidly. Norris' results (37) indicate that the recoiling is not due to the superficial protoplasm or plasma membrane alone. Elasticity can also be demonstrated within the cell by inserting a nickel particle and attracting it by means of an electromagnet. On breaking of the current, the particle is moved back by the elastic protoplasm (36).

Pfeiffer (38, 39, 40) found evidences of elasticity in the protoplasm of certain plant cells which was removed and subjected to various known pressures in a deformation apparatus. The fluid protoplasm was sucked into a capillary and its rate of induced flow was measured on an attached manometer. Plotting of the rates of flow against the applied shearing stresses showed an anomalous viscosity for protoplasm since the values obtained decreased as the rates of shear increased. In true liquids the viscosity remains constant as the pressure varies. The effects of temperature change and of several chemicals were also studied.

By means of a unique method, Norris (37) measured the elasticity of protoplasmic strands of a myxomycete. Using a pair of microneedles, the flexure of one showed the force required to stretch a given strand. The elastic modulus was found to be about 9.0×10^4 dynes per sq. cm. at room temperature for length increase up to 20 per cent. Tests with saponin indicated that the elastic properties were not confined to the superficial protoplasm. Chloretone stopped streaming of the protoplasm but had no effect on its elasticity. The latter finding is of special interest when compared to Marsland's results noted above.

Plasma membrane.—Investment of the cortical protoplasm by a thin elastic membrane apparently obtains for all cells. This membrane has frequently been confused, however, with the cell wall, vitelline membrane, pellicle, and other extraneous coatings (3, 4). The latter may be completely removed without affecting the integ-

rity of the cell but the plasma membrane is indispensable (3, 4, 5, 7). The extraneous envelope of mature, unfertilized sea urchin eggs can be mostly removed by vigorous shaking in sea water, then in an isotonic sodium chloride solution. When transferred to an isotonic solution of calcium chloride and mounted in a hanging drop over a microdissection chamber, some of the eggs may be seen adhering to the coverslip due to a gelatinous residue still surrounding them. An adhering egg may be pushed out of this gelatinous covering by means of a blunt needle. It may then be rolled about freely or dissected into spherical drops so long as the membrane remains intact. When the membrane is torn by a needle point the protoplasm coagulates. If, however, the tearing is made in an isotonic sodium chloride solution a wave of disintegration starting at the tear travels over the surface and converts the plasma film into minute droplets, whereupon the protoplasm flows out and disintegrates in the sodium chloride medium. A striking demonstration of an induced fluid behavior of the plasma membrane was made on these eggs freed of their extraneous coats in an isotonic calcium chloride solution. With a micropipette an oil drop was placed upon the membrane surface to which it adhered. The egg was then carefully pushed along by a blunt needle. Membrane currents were thus induced which at times flowed in opposite directions such as actually to shear in two the adhering oil drop.

Results of some modern researches tend to show that the plasma membrane, as a physically differentiated structure, is comparable with the membrane of the contractile and food vacuoles and with the tonoplast bounding the vacuole of plant cells. Plowe (41) observed the merging of the tonoplast with the ectoplast which showed their physical similarity. In many protozoa the contractile vacuole, preceding its discharge, fuses with the so-called *papilla pulsatoria* which evidently includes a plasma membrane; and the formation of a food vacuole, as in an ameba, directly identifies its vacuolar membrane with the plasma membrane (21, 42, 43, 44). These physical similarities in various protoplasmic membranes obviously leave out of account certain inherent differences that have been shown to exist. It is now generally agreed that permeability is controlled primarily by the plasma membrane (12, 45, 46, 47). Also, the accumulation of solutes, such as chloride in the cell sap and the occurrence of bioelectric potentials depend mainly on differences between the outer (ectoplast) and inner (tonoplast) mem-

branes (45). Scarth, Levitt & Siminovitch (48) found evidence also of a superficial lipoid film covering the plasma membrane. The film substance was highly surface-active, spreading rapidly over the surface of an applied oil drop. This sort of lipoid film, if of common occurrence, might account for the induced shearing in two of an adhering oil drop, as noted above, and may relate to the superficial lipoid monolayer described by Schmitt (49, 50).

Physical Structure

Finer protoplasmic structure.—The diverse physical properties of protoplasm point first of all to its physical differentiation. This initial emphasis on protoplasmic differentiation follows also from obvious comparisons between the heterogeneity of the protoplasmic unit or cell, even in its primordial stage of least differentiation, and the relative homogeneity of other physical systems. Correspondence between diverse properties and structural differences was emphasized, and sometimes overemphasized, in the formulation of the early theories of protoplasmic structure. These theories were mostly based on the results of microscopic observation, especially of fixed and stained material. Thus they incorporated the microscopic granules, globules, rodlets, or fibrils as essential components of protoplasmic structure. Since these visible inclusions of protoplasm are demonstrably not indispensable to its continued life and reproduction, its essential structure is evidently a finer structure that forms an integral part of the hyaline ground substance. The well recognized colloidal properties of protoplasm, including reversible gelation and elasticity, identify for it a colloidal structure and these properties have been demonstrated for the hyaline protoplasm (7, 36).

The elastic property of protoplasm would indicate that its finer ultra structure is essentially a framework (36) although elasticity has not been clearly demonstrated for protoplasm in a highly fluid state. Harvey & Marsland (51) found evidence, however, of a continuous structure in the protoplasm of *Amoeba dubia*. When centrifuged at high speeds, the inclusions were observed to move in jerks through all parts of the cell. They concluded that "the discontinuous movement must be due to a structure in the cytoplasm," possibly representing local sol-gel transformations. Evidence of a structural framework was reported by Banga & Szent-

Györgyi (52). Upon treatment of kidney tissue with a weak saline solution, a third of the total proteins dissolved out leaving "the solid edifice of the cell." Fibrous proteins composed this edifice, as shown by the intense birefringence of flow. The globular proteins went into solution. This essentially confirmed earlier findings of Bensley (53) that the gel and fiber forming constituent (plasmosin) of the protoplasm resisted saline solutions and the cells retained their characteristic form. Chemical concepts of cytoplasmic structure are recounted in a very recent paper by Bensley (53a). Schmitt, Bear & Palmer (54) demonstrated by optical methods an oriented lattice or cytoskeleton in neurons, which might be regarded as an organized complex of giant molecules. It was also suggested that this sort of cytoskeleton might represent the physical basis of polarity in cells and of axes of symmetry in the developing embryo (49). Pfeiffer (39) attributed contractility of the protoplasm of plant cells to a fibrillar complex of polypeptide chains. This complex accounted for an observed elasticity characteristic of non-Newtonian fluids and it exhibited many-colored interference veins parallel to the axis of flow. Baitsell (55) regards the evidences of a structural pattern in protoplasm to indicate basically a crystalline pattern such as obtains in inorganic solids. This would seem to carry the analogy too far. Concepts of a crystalline structure, along with those of an irreversible framework tend to leave out of account the reversible gelation changes in protoplasm which appear to be among its most salient properties.

Other physical systems.—Protoplasmic properties and structure have related the animate and inanimate worlds much as the cell united the kingdoms of plants and animals. Picken's extensive review (56) on the finer structure of biological systems abundantly illustrates these relationships in a variety of plant and animal cells and cell products. Uraguti (57) demonstrated a formation of concentric rings in plasmodium protoplasm, induced by applying or injecting solutions of various metallic nitrates, that strikingly simulated the appearance of Liesegang rings in gelatin. Evidences of common denominators in living and other physical systems have now reached proportions such as amply to justify the assumption that comparable phenomena in the two systems may be referred essentially to the same physical conditions. Thus, much the same structural conditions presumably

obtain for reversible gelation or for elasticity in protoplasm as in other physical systems. Also, the formation of membranes and of vacuoles in plant and animal cells apparently has its counterpart in the origin of similar structures in various colloidal solutions by the process of coacervation (58, 59, 60, 61).

Under given conditions, particles of some colloidal solutions tend to aggregate but do not come into contact. Bungenberg de Jong (58, 59) attributes their aggregation to mutual electrostatic attraction and their repulsion as due to solvation. Upon sensitization of a lyophilic sol, such as gum arabic, with alcohol and addition of a neutral salt, the particles aggregate into globules or form other structures, depending on the concentration of the colloid, the pH, the presence of other solutes, etc. The internal state of the globule varies with respect to both the effective attraction and the repulsion among its constituent particles. The addition of substances altering either set of forces will thereby change the solvate content. A vacuole may appear in the globule due to the formation of a vacuolar boundary of oriented molecules. Molecular orientation also accounted for the fusion or nonfusion of vacuoles. or of the globules themselves. Network structure, as well as vacuoles, could be induced in gelatin coacervates, their nature varying with the tempo of gelation and size of the globules. Slowly gelating globules, if small, gave a homogeneous gel; if large, the gel was vacuolated. Globules that gelated rapidly formed a network structure.

In the coacervates just referred to, the sol particles were essentially spherical so that their aggregates were also spherical. The aggregation of elongate particles, however, tends to form naviculate structures or tactoids (1, 62). Bernal & Fankuchen (63) have investigated the modes of aggregation of the particles in solutions of tobacco mosaic virus. In a concentration gradient, they observed that the particles were disoriented in low concentrations. At higher concentration, orientations appeared in the sol that passed continuously over into an oriented gel. Below a concentration of 4 per cent the sol was isotropic but showed flow birefringence. The gel was doubly refractive with regions that showed the particles parallel. The forces maintaining the particles equidistant and parallel were probably due to ionic atmospheres. Thus the orienting forces of the elongate particles exceeded their thermal energy. This excess effected final equilibrium. It was fur-

ther observed that when streaming through a capillary the rod-like virus particles oriented parallel to the current. When the flow was stopped, the particles aggregated to form many tactoids, conforming to forces acting tangentially and at right angles to the long axis of the particles and of the resulting tactoids. On jarring the container the tactoids came into contact and arranged themselves so as to form a continuous network throughout the medium, thereby producing a reversible gel whose elastic and birefringent properties were evident. It was suggested that a like behavior could account for gel formation generally.

Langmuir (64) regards tactoid formation and coacervation to be essentially comparable phenomena, In tactoid formation such as in thixotropic sols whose particles have like charges, the aggregation of the particles could be attributed to the Coulomb attraction between the particles and the oppositely charged ions, without requiring the long range Van der Waals forces. The Coulomb attraction would actually give an excess of attractive force which would have to be balanced by the dispersive action of thermal agitation and a decrease in charge on the colloid particles. "The assumption of a definite ζ potential, rather than a definite charge on the micelles, gives automatically just such a decrease in attraction." In the coacervation of particles oppositely charged, such as of dilute gelatin (positively charged) mixed with dilute gum arabic (negatively charged), the electrostatic fields and the charges on the particles increase as the concentration of particles increases. Thus the aggregation of particles can be accounted for but a repulsion force is necessary to prevent their coming into contact. Langmuir favors Bungenberg de Jong's (59) assumption that the increasing electrostatic field of the aggregating particles would tend not only to draw the particles together but also to draw between them dipole water molecules so that the resulting solvation would keep the micelles apart. Freundlich (62) points out that electrical forces alone, or along with hydration, may account for both the attraction and repulsion of the particles.

It would thus appear that the visibly reversible properties and structure of these coacervate and tactoid systems may be referable to the interaction between the colloidal particles and their ionic atmospheres as well as between the particles themselves. Since the physical forces involved, such as the Coulomb attraction and like or unlike particle charges, obtain also in other physical systems,

evidently the kinds of structure here formed, including globules, films, vacuoles, tactoids, and framework, relate more to differences resident in the particles concerned and in their ionic atmospheres. The role of other physical factors is also evident, notably the concentration of particles, the pH, and the orientation of molecules and of micelles.

Taking into account the behavior and products of these two physical systems, of which the striking similarities must greatly outweigh their differences, it seems reasonable to assume as a working basis that similar physcial conditions will come to be adequately demonstrated in the reversible properties and structure of protoplasm. Modern lines of research point clearly toward that prospect, but only brief reference to some of these will be made here. Freundlich (1, 62) has described and illustrated coacervate and tactoid formations and other properties of sols and gels that relate to protoplasmic structure. Schmitt, Bear & Palmer (54) identified by x-rays concentric layers of oriented lipoid and protein molecules in the myelin sheath (65). Schmitt (49, 50, 66) has described also other protoplasmic patterns and has indicated their significance in morphogenesis. These involved a protein cytoplasmic framework and demonstrable molecular orientations in the plasma membrane which could be factors in cell shape and cell movement. Thus a zipper action between adjacent cell surfaces might account for foldings during embryonic development (67).

Frey-Wyssling (68) has urged that our concepts of the structure of protoplasm must correspond with its physical properties, such as rigidity, shape constancy, tensile strength, elasticity, flow, plasticity, stickiness, anisotropy, contractility, thixotropy, imbibition, and semipermeability. To account for these properties as well as for finer details of metabolism, the classical concepts of a dispersoid (dispersed phase and dispersion medium) have to be abandoned. In its stead is proposed a reticular structure of molecular dimensions comprising a protein fibrillar framework to which lipoids, phosphatides, water, and inorganic cations and anions are attached or may be contained in the substance of the intermicellar spaces. Thus the framework and intermicellar substance are mutually interpenetrating as two coherent but immiscible phases.

Other recent analyses of protoplasmic structure include especially the contributions of Sponsler (69, 70, 71) and of Wrinch (72, 73, 74). These report the results of computations based on the

known molecular composition of the cell. It is there assumed that the atomic radii and valence angles and the kinds of valencies between atoms, molecules, and micelles obtain in protoplasm as in other physical systems. Thus dimensions and forces known for the latter are inferred for the former. The concepts of protoplasm structure thus derived by methods of computation appear to have much in common with those based more directly on observation and experimental results.

The foregoing accounts on properties and structure of protoplasm and of other physical systems have brought into focus certain parallelisms between animate and inanimate nature. Knowledge of their common denominators, which bespeak a common origin, is the basis for understanding their differences. These differences are real and apparently extreme, But there are also gradations of difference within inanimate nature as well as within animate nature, with parallels becoming more and more evident especially between the upper limits of the former and the lower limits of the latter. Thus the reversible properties described for coacervate and tactoid formations may have much in common with those recounted for protoplasm. The structure which these properties suggest, since they are reversible properties, is obviously not a fixed and irreversible structure. A protoplasmic framework, however conceived, must be such as to follow for reversible gelation changes.

Accordingly, the sol-gel transformations that appear during ameboid locomotion, protoplasmic streaming, and cell division must surely involve transformations also in any protoplasmic framework. Likewise, the appearance and disappearance of the nuclear membrane, the amphiaster, and all ciliary structures, as occur with each cell division and during other cell cycles, evidently also involve sol-gel transformations and thus would require a reversible protoplasmic framework.

The mechanism described for coacervate and tactoid formations would seem to suffice for reversible structural differentiations in protoplasm, including a reversible protoplasmic framework. Assuming for the living hyaline protoplasm the occurrence of both globular and fibrous constituents, such as the analyzed globular and fibrous proteins, then apparently contractile vacuoles, vacuolar, and other reversible membranes may come to be adequately

accounted for. Correspondingly, a reversible behavior especially of elongate constituents, similar to that described in tactoid formations, may likewise bring within range of our human understanding not only the reversible differentiation of the amphiaster and of ciliary structures but equally so the formation, dispersion, and reformation of an essential protoplasmic framework.

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PHYSIOLOGICAL ASPECTS OF GENETICS

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Functionally, the gene may be defined as a self-perpetuating unit of specific synthesis in biological systems. Thus the mutant types of organisms give material for the comparative study of intermediary metabolism as well as presenting their own peculiar puzzle—the nature of the change leading to the aberrant development. The recent advances in the understanding of nucleoproteins brighten the hope that, by the study of the chemistry of the chromosomes and related cytoplasmic nucleoproteins, more direct insight may come to these problems.

Techniques.—For the problems of intermediary metabolism, Beadle & Tatum (1) have devised a technique which makes possible the detection of mutants for specific biochemical syntheses. In the mould *Neurospora*, following irradiation of the spores and their establishment on a medium containing all the known growth factors, the individual strains are tested for growth on a minimal medium, in which the normal fungus can grow. The mutants requiring now the addition of growth substances to the medium are tested with the individual factors, and the required substance thus identified. Another technique for study of chemical behavior has been applied to yeast by Stier & Castor (2) in which survivors in a population subjected to a respiratory poison are found to lack the sensitive respiratory system—a selection of mutants already existing, or an adaptive process of some type.

Stadler (3) has adapted plant tissue culture techniques to the study of anthocyanin synthesis in maize. Thus the opportunity is taken to identify, by using different substances in the media, the specific biochemical step affected by the mutants under study.

For the direct study of native chromosomes, Buck & Melland (4) describe procedures for quantity isolation of oriented salivary gland chromosomes (designed as material for the study of the x-ray diffraction pattern of the chromosomes, the attempt so far having been unsuccessful). Laskowski (5) has isolated nuclei which respire from bird erythrocytes by hemolysis with lysolecithin. Mirsky &

Pollister (6) have developed methods which permit study of the nucleoproteins of tissue nuclei in the native state. Extracts made with sodium chloride solutions of one or two molal concentration from fresh tissue at low temperatures give on centrifugation a supernatant fluid from which the fibrous nucleoproteins can be precipitated by dilution of the saline solution. These nucleoproteins are proved to have their source in the nuclei of the extracted tissue. Thus the comparative analysis of the nuclei of different tissues is being undertaken.

Cytochemical techniques are being increasingly applied to the identification of the substances which form the chromosomes. The different techniques permit most certain conclusions when reinforced by each other. The optical methods, considered by Caspersson (7), identify substances by their intrinsic properties; ultraviolet absorption spectra of objects as small as 0.5μ can be taken with a high degree of accuracy. In combination with the digestion of the structural links by specific enzymes as used by Mazia (3) and differential staining plus enzymatic digestion as used by Schultz (3), the prospect is good for the precise localization of the different chromosomal materials.

Nucleoproteins, viruses, and chromosomes.—The analogies between viruses and genes, originally made on the formal basis of their self-reproducing capacity, now have a substantial basis in the association of nucleoproteins with both types of activity. The isolation and identification of an increasingly large group of viruses has now permitted comparative analysis of the composition of related strains (3, 8, 9, 10, 11). Six serologically closely-related strains of tobacco mosaic virus proved to be very similar in their aromatic amino acid content (3.8, 4.5, and 6 per cent of tyrosine, tryptophane, and phenylalanine, respectively). Three other strains, more distant serologically, showed significantly different values, the rib-grass strain having 6.4, 3.5, and 4.3 per cent, while two cucumber viruses had 3.8, 1.4, and 10.2 per cent, respectively of tyrosine, tryptophane, and phenylalanine. The nucleic acids of all these strains are constant so far as tested, and it is indicated that the specific properties of the virus are determined largely by their protein moieties, somewhat analogously to the nucleoprotein respiratory enzymes. It is pointed out that these strains are to be regarded as involving mutations, and that the changed amino acid constitution implies a deep seated change in the molecule.

This would be consistent with the idea that the mutations are changes associated with errors in the reproduction of the molecule and not simple rearrangements of constant constituents.

The fundamental problem of the nature of self-reproduction in the virus has been attacked by the preparation of derivatives (phenylureido and acetyl) of the viruses, inoculation of plants with the derivatives, and analysis of the type of virus produced (12). As a result it turns out that virus activity is not decreased by blocking 70 per cent of the amino groups and 20 to 40 per cent of the phenol groups. Since the virus produced by the derivatives is identical with the original, it follows that the pattern is determined by groups not involved in the substitution. Experiments on the growth of the virus in the plant (13) give indication that the synthesis of the specific grouping may be a stepwise affair, since the high molecular weight protein isolated in the early stages has a lower specific activity than that from older leaves. The radioactive isotope of phosphorus has been used (14, 15) and it is found that there is an exchange of phosphorus in the synthesis of virus. The interesting experiment (15) of inoculating plants with virus-containing radioactive phosphorus proved inconclusive as it was uncertain how much radioactive phosphorus entered the plant as virus, how much as degeneration products.

The viruses are ribonucleoprotein in nature, the chromosomes desoxyribonucleoprotein. The much discussed question of the nature of the bacterial nucleus, and indeed whether one exists, studied by Knaysi (16) and Robinow (17) finds an answer for certain cases in the presence of a desoxyribonucleoprotein body undergoing a fission at cell division. Thus the correlation of bacterial reproduction with that of higher organisms is achieved. Actually in the case of the virus nucleoprotein, one of the differences between the two types of nucleic acid—the high capacity of the thread molecules of desoxyribonucleic acid to polymerize—seems to become blurred with the new work on the thread molecular nature and the spontaneous birefringence shown by the native virus nucleic acid (18).

Information of the same nature as that recounted for the viruses is obtained for the chromosomes by the combination of techniques outlined above. Reviews of a cytological nature have appeared (19, 20), and a summary (21) may be mentioned. Most precise is the information of the structure and composition of the giant chromosomes of the salivary glands of the Diptera.

Much discussion of the cytological structure of these chromosomes, such as that by Metz and Painter at Cold Spring Harbor (3), has led to the view that their giant nature is due to a process of submicroscopic dimensions, whether growth or splitting, is not clear. The nucleoprotein bands are highly specific in form and behavior and connected in a fiber by protein interband spaces. The analysis of ultraviolet absorption spectra has led Caspersson (22) to the differentiation of three protein components, recognizable by the nature and content of their aromatic amino acids: a protamine-like component, with a nonspecific absorption in the far ultraviolet; a component with the usual aromatic amino acid absorption band at 2800 Å; and another component localized in certain regions, with a band around 2900 Å. These three types are also perhaps discernible in the bands, but the relatively enormous 2600 Å absorption band of the nucleic acids present there makes analysis difficult. The interband spaces stretch considerably, and microdissection experiments (23) show 300 per cent stretch before breakage, a fact indicating a degree of intramolecular folding. The results of the enzyme analysis complement the absorption data: the structural skeleton of the chromosome is protein (trypsin digestible) according to Caspersson (24) and Mazia (3); the nucleic acids may be removed without structural disturbance [Mazia (3) and Frolowa (25)]; peptic digestion in experiments of Mazia removes a "matrix" (3), containing, therefore, the more acidic proteins. The triphenylmethane dye fast green combines with most of the proteins but not with the protamines and serves in combination with the enzymatic analysis to localize the components (3); the non-staining "protamine" is the structural element; the bands contain proteins which combine with the dye, as do rather specific "puff" regions and the "matrix." The orientation of the nucleoprotein molecules in the chromosomes has been the subject of controversy as different techniques have led to different results. The presence of low polymers of the nucleoproteins in the salivary gland chromosomes has been under discussion by Mazia and Caspersson (3, 26, 27).

Kodani (28) has attempted, by observing the stages of degeneration of structure in urea-alkali treated chromosomes, fixed at different times, to make deductions concerning their ultimate structure. The hazards of such deductions in systems whose structural phases may separate on denaturation are great, and it is

evident that only conclusions confirmed by other techniques can be relied on. The effects observed arise from the splitting of the nucleic acid off the protein (denaturation), solution of the nucleic acid in the alkaline medium, and its diffusion in a heterogeneous system bounded by the membrane, precipitation or preformed, around each of the original four strands of the chromosome. The occurrence of this membrane is a point of interest [also discussed by Painter (3)], as it reinforces the developmental evidence of a four strand structure for these chromosomes. The various types of picture are partially dependent on the initial variability of the chromosome, according to Painter (3), and upon the strength of the solution. The successive interpretations of the facts have not found ready acceptance; the novel recent one of a coil of swollen chromomeres as composing each band has been particularly difficult due to the drastic experimental procedure.

The salivary gland chromosomes may be taken as an example of the chromosomes of the interphase nucleus. Associated with specific regions in the chromosomes of the interphase nuclei, the nucleoli are found on spectrophotometric analysis (29, 22) to contain proteins and nucleic acids of the ribose type. The presence of proteins has also been shown by other cytochemical techniques (30). The ribonucleoproteins have been shown to be correlated in their occurrence in the cytoplasm with active growth and protein synthesis (31 to 36), and their presence in the nucleolus has led to attempts to interrelate the two systems. The presence of the ribose type of nucleoprotein in the chromosomes of the interphase nucleus is not certain, although the enzyme ribonuclease has been shown by Schultz to loosen structural links in the salivary gland chromosomes (3); however, the action of the enzyme itself is not yet sufficiently well understood. In any case the macrochemical analyses of sperm have shown that the chromosomes in the inactive state contain little if any ribonucleic acid.

The reproduction of the chromosomes in the meiotic prophase has been shown to be correlated with an increase in the desoxyribonucleic acid content (37); and on their contraction to metaphase, a change in the proteins also has been discussed by Caspersson (22, 32). In his view, the breakdown of the complex proteins observed by Kossel in spermatogenesis is a phenomenon occurring at every prophase, with the converse elaboration of the complex proteins occurring at every telophase. Evidence consistent with

this rapid nucleic acid synthesis at mitosis, from the use of radioactive phosphorus as a tracer, has come quite recently (38, 39). In tumor cells with a high mitotic rate, isolated nuclei (Marshak) and desoxyribonucleoprotein (Brues *et al.*) show a high phosphorus turnover. In the resting nuclei, however, the desoxyribonucleic acid fraction itself shows a low turnover; in contrast a considerable exchange is shown by the acid-insoluble residue (nucleoprotein) when the nucleus is analyzed (38). It seems not unlikely that the phosphorus exchange in the resting nuclei is concerned with the ribonucleic acids and that the structural element involved is the nucleolus—a situation consistent with the growth of the nucleolus and its active function in the interphase nucleus. The recent cytochemical evidence of the presence of alkaline phosphatase (39a, 39b) in chromosomes and nucleoli is of distinct interest here.

Heterochromatin and its function.—The regions of the chromosomes that stain most deeply at interphase are called heterochromatic regions (40), and the very nature of the definition, when the stain used is the Feulgen reaction, implies a special relation to the desoxyribonucleic acid synthesis. In *Drosophilia*, the heterochromatic regions have been shown to affect the ribonucleic acid metabolism of the cell by way of the nucleolus (41), as well as the ribonucleic acid concentration of the egg cytoplasm (42). More recently the reaction to temperature of special segments of chromosomes in *Trillium* (43), in various other plants, and in the newt (44) have been considered (Darlington & La Cour) to give a new technique for the detection of heterochromatin. Kept at low temperatures during the proper stages, the regions which fail to contain the full amount of desoxyribonucleic acid at metaphase are considered heterochromatic. There is some doubt still (45) as to the exact identification of the segments in question with the interphasic chromocenters which form the basis of the original definition of heterochromatin; the possibility is not excluded that the older description of the same phenomenon (prominence after such treatments of the constrictions which frequently neighbor heterochromatic regions) may be the correct one even if the association were demonstrated. If the correctness of the identification is assumed, the particular interpretation chosen ("nucleic acid starvation of the heterochromatin") is not easily to be reconciled with the view, to which Darlington (21) also subscribes, that the heterochromatic regions are centers of nucleic acid synthesis, either directly or indirectly as sources of protein precursors.

In the salivary gland chromosomes the proteins associated with heterochromatic regions, usually nucleolar and not fibrous in nature, show the conspicuous shift of the aromatic amino acid ultraviolet absorption band referred to above, to 2900 Å from the usual 2800 Å. This has been interpreted (22, 32) to indicate their high basicity and historic nature, but recent reports (6) that the absorption band of various histones is at 2800 Å reopen the question. The substance responsible for the 2900 Å band is considered by Caspersson to be histone in nature, to be synthesized by the heterochromatic regions to form the nucleolus, and is regarded as a precursor of the cytoplasmic nucleic acids of the cell. Since these in turn are associated with rapid protein synthesis (31, 33, 34, 35, 36) the argument is made that the basic cytoplasm of the cell is the product of heterochromatin (32). The original view, based on various types of genetic evidence, was that these were "inert" regions (40, 47). It was later suggested that a nonspecific action would give the same type of genetic results and account in addition for the relation to nucleic acid metabolism (42, 46). The question that is raised, however, is whether this nonspecific function is common to all genes or peculiar to heterochromatin ("degenerate" or "primitive" genes).

The activity of heterochromatin in plants has received further study. The B type chromosomes in maize may be accumulated as supernumerary chromosomes, as many as thirty-four having been seen in one plant. Until these very high numbers are reached, no particularly untoward effects have been observed (48). The accumulation of these chromosomes in natural populations has been used as an argument for their having some function of selective value (49). New studies (50) show that the accumulation is due to a failure of separation of the sister chromatids at the second microspore division, while the gamete has either two B chromosomes or none—the result of abnormal behavior of the centromere. In *Sorghum*, B type chromosomes have been found even more aberrant (51): they are eliminated in the root tissue and persist only in the shoot. (This may be compared with "diminution" in *Ascaris*, etc.) A closer study (52) of the pollen grain divisions has led to the interesting discovery that in pollen grains with extra B chromosomes, the B types go into the generative nucleus at the first division, following which the vegetative nucleus undergoes a series of additional polymitotic divisions. This is interpreted as an effect, similar to those discussed above, of the heterochromatin on the

cytoplasm, exerted here between meiosis and the first mitosis of the pollen grain.

In both these cases, and in others, there is a tendency of the heterochromatin towards slower division. In the intranuclear chromosome multiplication of differentiated tissues, the "endomitosis" discussed by Berger (3) and Geitler (53), a similar phenomenon appears, noted by the latter as the failure of separation of the heterochromatic chromosomes, In the *Drosophila* nurse cell endomitoses, where the situation can be more closely analyzed, the failure of separation seems to be associated with a slower reproductive rate of the heterochromatic regions [Schultz (3)].

The correlated genetic, cytological, and cytochemical studies of the interaction between heterochromatin and euchromatin in *Drosophila* give the most concrete evidence of the nature of the function of heterochromatin in genetic terms. The increase in nucleic acid concentration on salivary gland chromosome bands juxtaposed to heterochromatin in the variegational translocations of *Drosophila* (42, 46, 54) is correlated with mild genetic effects on the genes at the loci in question. [See also Schultz, Cole & Sutton, and Muller (3).] Severer deviations from the normal accompany the assumption of a definite heterochromatic form, and in the most extreme cases (apparent genetic deficiency) the band in question can no longer be found. The interpretation of the results in terms of a lowered reproductive synthesis, correlated with the assumption of the heterochromatic nature [Schultz (3, 46)], derives from the cytological observations of the behavior of these heterochromatic regions in endomitosis. Worth noting is the implication of a relation between the reproduction of the genes and their specific activity, in terms of an upset nucleoprotein metabolism.

Chromosome behavior.—Genetically controlled abnormalities of chromosome behavior offer interesting material for the chromosome physiologist, presenting as they do a sample of the complex processes that are to be unravelled. A mutant in barley is reported (55) whose pollen mother cells fuse to form a plasmodium, in which as many as 112 pairs of chromosomes may appear on a single metaphase plate but the spindle is nevertheless of normal length. In maize, a chromosome has been found (56) which undergoes a preferential segregation in megasporogenesis, passing to the egg twice as frequently as its normal homologue. A slight temperature effect

has been demonstrated on the course of meiosis in the sex-ratio mutant of *Drosophila pseudoobscura* (57).

Alkaloids, centromeres, and centrosomes.—The role of the centromere, its function as a special type of gene, and the assumption by the free centromere of centrosomal duties in the aberrant sperm of prosobranch molluscs (58) have been discussed in various connections (59, 60, 61, 62). In barley evidence is given that in addition to inhibiting spindle formation, colchicine treatment causes transverse division of the centromere (63). The interrelation is of interest in the consideration of the results of colchicine treatment of *Fritillaria* at early meiotic prophase, in which the agent suppressing the spindle at later stages influences a change in chiasma distribution on the chromosomes, a fact which is interpreted as an effect on the centromeric control of this distribution (64). In *Antirrhinum*, univalent formation following colchicine treatment has been reported and interpreted as due to a decrease of chiasma frequency (65). It is not unlikely in view of the results on the treatment of grasshoppers with colchicine (67) that in animals, although the spindle is inhibited by colchicine, the centrioles behave towards the drug as do the chromosomes; they divide almost as usual, and as the treatment is on spermatocytes, polyploid sperm formed are biflagellate and presumably nonfunctional and have a tail from each centriole. The reaction seems a general one since no polyploid races have been obtained from colchicine treatment in animals. To the substances which induce polyploidy the alkaloid sanguinarine has been added (66).

Chromosome rearrangements.—The consensus of opinion at the Cold Spring Harbor Symposium on Genes and Chromosomes (3) was that the immediate effect of an ionizing radiation on a chromosome is the production of a "potential break"; the rearrangements are the result of new types of reunion, the single breaks the result of failure of restitution. The detailed implications of the analysis are various, depending on the arrangement of the chromosomes in the particular nucleus, the time of division, the stresses imposed by chromosome coiling, and other such variables. Thus a lower frequency of breakage in acentric chromosomes of *Tradescantia* and of chromosomes with multiple aberrations, is interpreted (68, 69) as related to the role of the centromere in maintaining chromosome polarity. A new type of dosage-effect curve

for single breaks, with an exponent higher than one, has been obtained (70, 71). The suggestion made is that, in addition to the formation of the potential breaks, an effect of the x-rays on the restitution process is involved, such that at higher dosages fewer restitutions take place and more single breaks remain.

Neutron and x-ray efficiency for chromosome breakage have been compared for both *Drosophila* (3) and *Tradescantia* (72, 73). In the huge chromosomes of *Tradescantia*, the concentrated ionization of the neutron is more efficient; in the small *Drosophila* chromosomes, x-rays are somewhat more efficient and the concentrated neutron-produced ions are wasted. Thoday infers that more than one ionization is required to break a *Tradescantia* chromosome, hence the greater efficiency of the denser neutron ionization. The relative efficiency of neutrons and x-rays has also been studied for the different stages of the mitotic cycle in *Vicia* (74). There is a maximum neutron efficiency in early resting stage and a decrease as prophase is attained. It does not seem unlikely from the time relations that the initial maximum may be occurring, not in the resting stage, but during the telophasic reconstruction, when the uncoiling of chromosomes would provide a critical period in which movement is going on in a still unattenuated chromosome fiber, where the dense ionization could be more effective in breakage.

Swanson (75) has reported a comparison of the effects of ultraviolet and x-ray treatment on *Tradescantia* pollen tube chromosomes. Deletions (achromatic lesions) and single breaks were found following ultraviolet radiation, but as in maize and *Drosophila* [Stadler and Muller (3)] there is a deficiency of translocations compared to x-rays. An interesting datum is the decrease of effectiveness of the ultraviolet treatment as prophase advances.

The effects of x-rays have been attributed to an initial effect on chromosomes, which is displayed inthe following mitotic cycle. Bishop (76) reports effects following irradiation of the primordial germ cells in grasshopper embryos which only become manifest some cell generations later at meiosis. Breakages appear between chiasmata and the centromere in preferred regions of special chromosomes. It seems possible to the reviewer that the achromatic lesions seen in the spermatogonia (since translocations or inversions were not found in sufficient quantity) may represent the changes which later break at meiosis under the tension of ter-

minalizing chiasmata. In this way the effect may be accomodated within the existing beliefs.

Mutation.—The present status of the mutation problem is reviewed in the Cold Spring Harbor Symposium volume already referred to. That mutations have their origins in intramolecular rearrangements is the preferred view. The difficulties arise in the recognition of "true mutations," as contrasted with submicroscopic deficiencies or rearrangements. For endosperm deficiencies in maize, the data and analysis have been presented (77) demonstrating that the course of the curve for the relative efficiency of the different wave lengths of the ultraviolet spectrum approximates the absorption spectrum of nucleic acid. The importance of the nucleic acid of the nucleoprotein molecule is not unexpected, since chromoproteins can be denatured by light absorbed by their prosthetic groups (in this case nucleic acid).

Pontecorvo (78) has made an analysis of the x-ray induced dominant lethal mutations in *Drosophila*, showing that the transition fron a one-hit to a two-hit dosage effect curve for these types results from the inclusion of lethal effects from both one-break and two-break chromosome rearrangements.

The problem of the relation of bacterial mutations to those of higher organisms has been studied by observing the rate of production of mutations by x-rays in bacteria; the question at issue is whether the orders of magnitude are the same, which turns out to be the case apparently (79). Statistical techniques for dealing with low mutation rates for tests of significance have been discussed (80, 81).

A comparative study of x-ray and ultraviolet induced mutations in *Neurospora* has been carried out (82, 83). The results so far are similar to those in maize and *Drosophila;* chromosome rearrangements are detected in this form by the production of aborted ascospores, which are found in the x-ray but not in the ultraviolet series.

Spontaneous mutation occasionally reaches appreciable rates in special stocks; one such has been analyzed in *D. melanogaster* (84), in which the high rate is traced to a factor in the third chromosome (dominant) with an influence in X also possible. A case of variegation due to an unstable gene in *Portulaca* has been found to have a decreased mutation rate at high temperatures (85), contrary to the usual relation between temperature and mutation and somewhat reminiscent of the effect of temperature on *Drosophila* variegation.

Paths of synthesis in mutant types.—The three different biochemical mutant types of *Neurospora* thus far described (1, 86) differ from the normal in their respective capacity to synthesize vitamin B_6, the thiazole half of vitamin B_1, and *p*-aminobenzoic acid. The "amino-benzoicless" mutant has been tested with a variety of substances structurally related to *p*-aminobenzoic acid, in the hope of identifying the step in the synthesis of this substance that is blocked in the mutant. Tests were also carried out of effects of the related substances on the inhibition of *p*-aminobenzoic action by sulfanilamide. In this way both the possible precursors were looked for, and a possible role in the competitive reaction with the sulfanilamide (the reaction testing utilization of *p*-aminobenzoic acid) could be evaluated. Substances lacking the nitrogen in the benzene ring were completely inactive, and the slightly active substances gave no evidence of being concerned in the reactions of normal utilization of *p*-aminobenzoic acid. Thus the conclusion is reached that none of the compounds as yet tested is concerned in the normal synthesis of *p*-aminobenzoic acid.

In albino maize, the process of carbohydrate synthesis is interfered with—there are no normal plastids. Success in culturing the otherwise lethal type has been attained (87) by the administration of sucrose to the tips of the leaves under proper conditions. By analogy with the *Neurospora* mutants, the process blocked, whose synthetic deficiency is restored by sucrose, is the synthesis of sugar. What is deficient in albino maize, however, is the cellular condition for the proper differentiation of plastids. The analogy may be of use in considering the difficulties to be encountered in the use of these techniques.

Tests of the growth requirements of ten different strains of yeast are relevant here (88). One strain was found dependent on thiamin, four on inositol, all on pantothenic acid (growth being restricted without it), and on biotin (no growth occurring in its absence). Some strains exhibited growth effects of combinations of different vitamins, where singly no apparent effect was visible. The facts indicate a complex interlocking of the systems. An autotrophic strain of yeast has been reported (88a), employing the technique of Stier & Castor (2). These strains are material, apparently, for analysis of the *Neurospora* type.

In more complex organisms, effects on growth or differentiation accomplished by single nutritional factors are difficult to work out.

For *Drosophila*, the expression of a mutant type in which the growth of the antenna is inhibited has been related to the nutrition of the larva (89). The variability in expression turns out to be chiefly related to the changes in the yeast flora of the medium. Following this clue, it now appears probable that one of the effective factors is a higher vitamin B_2 requirement in the mutant than in the normal type for the growth of the antennal bud.

One of the goals of the studies of paths of synthesis is the identification of the enzyme systems involved. Comparative studies of the enzymatic activity of different strains of tissue are therefore of direct interest. Thus, a group of hepatomas, compared with normal and regenerating livers from the same strains, showed that almost each strain had a characteristic activity for the seven enzymes systems tested (90). Similarly, a difference between two strains of mice in the nuclease activity of blood and spleen, but not liver, was discovered as the result of an interesting analysis of the leucocytic response of two different leukemic types to injection of thymonucleic acid (91).

Antigens and genes.—The antigenic properties of cells and serum, related as they are to the specific configurations of the proteins and their prosthetic groups, are regarded as the material par excellence for the study of specific gene products. Irwin & Cumley have continued their studies of the segregation of cellular and serum antigens in the various dove hybrids (92, 93, 94). The serum proteins turn out to contain different antigens characteristic of the species as well as common antigens. The F_1 hybrids contain all the common components of the sera of their parents and all or almost all of the specific components. Backcross progenies show segregation of the specific serum antigens, which have been shown in certain cases to be independent of the erythrocyte antigens (95). In certain crosses (Ring dove by Pearlneck), the erythrocytes contain a new antigen not present in either parent —the well known "hybrid substance." This has been analyzed (96) into three separate antigens, two of which show complete linkage with certain of the Pearlneck characters. The third of the antigens shows loose linkage with other Pearlneck characters, and it is suggested that the substance or group of immunologically indistinguishable substances are produced by the interaction of various Pearlneck and Ring dove genes. Combined with the idea that antigens are "more or less primary products of the genes," the

results lead to the suggestion that the various genes within each of two species which interact to produce the third antigen are really duplicate genes. Alternatives involving longer reaction chains between gene and antigen are of course conceivable. The extraordinary diversity of the antigens in erythrocytes and the usual direct correlation between parent and offspring (which produce all the antigens that both their parents produce) are again illustrated, however, in the work on cattle (97). Thirty antigens have been detected; there are two possible cases of linkage and no interaction between genes to produce hybrid substances.

An allelic pair of erythrocytes, H_1 and H_2, and an independently inherited one, H_6, are described in the rabbit (98). The homozygotes and heterozygotes for these genes are distinguishable by the strength of the reaction of the cells in the homologous antisera,—the difference in titer being two dilutions. The interesting *Rhesus* antigen in man, shown to be the cause of such disturbances as erythroblastosis fetalis because of the passage through the placenta of its antibody into the circulation of the *Rhesus* positive fetus, has been found to be almost uniformly present in full-blooded American Indians (99). In one case of erythroblastosis fetalis, it has been possible to demonstrate the presence of the Rh antibody in the breast milk (100). In a study of the subgroups of the blood group A in man, the conclusion is reached that the group specific substance in saliva differs from that in serum (101). In view of the high species specificity shown by the usual tissue and serum proteins, it is interesting though not unexpected that study (102) of various insulins (those of beef, pork, sheep, bison, dog, and man) has shown that they are not distinguishable immunologically.

The similarity between the antigen-antibody reaction and the specificity of transplants has been discussed in these reviews previously. Gorer (103) has studied transplants of the stem-cell leukemias from this point of view, finding that susceptibility to the transplants depends on two unlinked dominant genes. One of these is almost certainly identical with a gene determining the presence of an erythrocyte antigen, the second possibly similar in nature. No effect of suspending the cells in immune sera on frequency of take was found, although *in vivo* the development of protective antibodies assumed major importance, causing re-

tardation of leukemic growth in some experiments and failure to take in others.

Specificity of incompatibility in plants.—The phenomenon of self-sterility in plants has long been recognized as possibly related to the specificities of transplants and to immunological reactions. Following this line of reasoning Lewis (104) has studied the effect of temperature upon the incompatibility reaction. The growth of compatible pollen tubes is optimal at fifteen to twenty degrees Centigrade in both the types of incompatible system: that determined by the genotype of the pollen grain (*Oenothera* and *Prunus* in this study) and that determined by the genotype of the parent (the heterostyled *Primulas*). The effect of temperature on the inhibitory process and on the growth rate of the pollen tube interacts in the incompatible types to place inhibition at different zones in the style, evidence confirming Emerson's thesis in *Oenothera*, that there is no specific inhibitory zone. At some temperature in *Prunus*, the incompatibility was broken down. In the heterostyled primulas, evidence is found for an adjustment of the growth rate of the pollen tube to the exigencies imposed by the different style lengths. The stronger inhibitory reaction at higher temperatures is interpreted as analogous to the antigen-antibody reaction.

The incompatibility of the "oppositional allel" type, in which the pollen tube grows only in a style containing a different allel than its own, is presented with an interesting situation in polyploids. Two of the allels are present simultaneously in the diploid pollen. In both pears (105) and *Petunias* (106) autotetraploids from incompatible plants have been found to give compatible pollinations. The interpretation offered (105) is, of course, that the compatible pollen tubes are those containing two different allels of the series. It has, however, been noted that with the stronger incompatibility reaction of *Brassica* (107) and of *Oenothera organensis* (105), the autotetraploids are still self-sterile. It has been reported that in certain plants the incompatibility can be broken down by treatment with α-napthalene acetamide (108). The effect is now reported to be due to a delay induced in the formation of the abscission layer at the base of the style, which gives the slower growing pollen tubes enough time, in weakly incompatible types, to achieve fertilization (108, 109). Self-sterility in the potato is

now reported to be due to another case of the "oppositional" allel system (110).

Tumors and tumor susceptibility.—It is difficult to test the theory that the differential reaction involved in tumor formation is of the nature of somatic mutation. An attempt in this direction has been made in the analysis of the rate of formation of tumors of the skin in mice after treatment with benzpyrene (111, 112). On the assumptions that the formation of a single papilloma involves originally a self-perpetuating change in a single cell, that this change is a recessive mutation, and that the effect of the benzpyrene is to increase the rate of this change, equations were derived which represent fairly well the data of papilloma formation and which demand a not unreasonable rate of mutation. In evaluating such attempts it is recognized that the fit of the data is consistent with the theory, but does not prove it.

Susceptibility to development of mammary tumors is now regarded as influenced by the interaction of estrogenic hormones of the "active milk influence," apparently virus like in nature, and of other genetic factors. Bittner (113) has reviewed the present situation, and from the analysis it appears that the hormones and the milk influence are influential in the development of the mammary glands, and the major genetic variable is concerned with the structure of the gland. This genetic susceptibility is influenced by the male parent as well as the female (114) and hence is chromosomal in nature. The extra-chromosomal influence has been found even more effective when ova are transferred from high to low tumor strains, or vice versa, than when the exposure is effected after birth by foster-nursing (115). The new characteristics acquired by the individuals transferred as ova are transmitted to their descendants. The active milk influence itself appears (116) to be a high molecular weight body, still active in filtrates or extracts of glycerine treated tissue, and not associated with the fatty fraction or the supernatant fluid following centrifugation. An apparently similar effect of foster-nursing to that described for the mammary gland tumors is now described for transplantable leukemias (117).

Complementary factors for the susceptibility to a sarcoma (118) and multiple factor inheritance of incidence of both spontaneous and induced pulmonary tumors (119, 120) are reported. Similarly, susceptibility to a transplantable melanoma is inherited

as a dominant in colored mice, but for the transplant to take in an albino, it appears that an additional factor must be present (121).

Comparative embryology of mutant and normal types.—The differential reactions between normal and mutant types sometimes involve specific reaction systems; occasionally they are general effects common to all cells. Thus careful studies of the growth rate of the Minute mutants in *Drosophila* as compared with the normal (122) show the relative growth rates of both types to be the same, but the Minutes grow more slowly and end up with smaller cells. Apparently the basic reaction involved is a retardation of cellular growth rate, mitotic and endomitotic, since both larval and imaginal growth is affected. In many cases, very diverse effects when analyzed are traced back to effects on a specific tissue system. Thus the lethal effect of the homozygous Creeper fowl, (123, 124, 125) earlier interpreted as involving a general retardation of growth, turns out to be due to an inhibition of the yolk-sac circulation and of various other derivatives of the splanchnic mesoderm, abnormalities which can be homologized with those that the heterozygote displays in milder form. On the other hand, the case of the homozygous yellow mouse, recently restudied (126), shows a lethal action which cannot yet be traced as a less severe process in the heterozygote. The cells of the embryo degenerate after the trophectodern of the blastocyst has come into contact with the uterine epithelium of the decidual crypt. When, by ovarian transplantation, the development of such embryos is observed in a normal uterus, an effect of the normal tissue on the lethal embryo is seen, resulting in an extra cell division and even successful implantation of the embryo.

A useful orientation in the experimental work on feather development is provided in a review by Lillie (127). The differential response of the red and the black melanophores to the steroid hormones has been studied (128); the differentiation of the red melanophores in skin explants is facilitated by the sex hormones, of which testosterone is the most effective. The black melanophores (both New Hampshire and Rhode Island Red) are increased in number by testosterone and estrone, inhibited by estradiol and olive oil. The two types of cell are distinct; the differential reaction occurs after the migration of the melanoblasts to the wing buds. Melanophores of these types, grown in tissue culture from neural crest

explants, differentiate and maintain their cell type (129). Transplanted into embryos, the frequency of takes decreases with the age of the culture and varies with the genetic composition of the host. The results from these and other experiments have been interpreted by Humm to indicate that the red and black melanophores are stages in the differentiation of one type of cell. They are, however, reconcilable with those of Hamilton, since according to him the differentiation into the two types takes place after the migration from the neural crest.

The development of the scalloped wing pattern in *Drosophila* has been a subject of discussion between Goldschmidt, according to whom the scallops are the result of lytic degeneration of portions of the wing, and Waddington, who believes they arise from abnormal folding of tissue with respect to a wing pattern previously determined. New studies of the early stages deemed critical give observations in agreement with Waddington's studies of the same stages, but certain features are interpreted as favoring the Goldschmidt interpretation (130). Experiments studying the effective period for altering the wing pattern by starvation (partial or complete) show the beginnings of determination of the scalloped pattern in the young larva (131). Defect experiments—operations on the wing at various stages of pupal development—are interpreted to give the results expected from Waddington's analysis of wing development (132).

A comparative analysis of the development of the structural mutants of the *Drosophila* eye has been carried out (133). The mutants depart in specific ways from the normal rule of facet development (orientation of the cells of the middle layer of the optic bud, with directed divisions to give rise to the retinulae and to the cone cells which invade the outer layer). In the mutant rough, a degeneration of cone and retinular cells takes place; in split, the division of the penetrating middle layer cells and their invasion of the outer layer is irregular; and in Glued, both the invasion and the two divisions are irregular.

A similar analysis of the bristle mutants has been carried out (134). The joint secretory activity of two sister cells form bristle and socket, respectively. The initial differentiation is inhibited by scute and hairy; an extra division into socket and bristle cell is affected by split. The arrangement of the two cells with respect to each other is abnormal, resulting in only socket and no bristle, in

Hairless and shaven, or in a change in bristle shape (Stubble). The growth of the secretory cells, which have large endomitotic nuclei, is affected in such small bristle mutants as spineless and morula; the secretion of the bristle is affected in various mutants that change bristle shape; and the final darkening and hardening of bristles are affected in still other mutants.

Correlating the approach of the experimental embryologist with the attack on these problems, Waddington has considered the pupal contraction following eversion of the imaginal disc as a developmental crisis, deviations from whose delicate adjustment are responsible for the characteristics of many of the striking morphological mutants in *Drosophila* (135). Similarly, the production of developmental abnormalities by x-radiation of the various developmental stages is restudied in an attempt to analyze the time of determination of the imaginal discs in *Drosophila* (136). The "phenocopies" are interpreted as initiated in development by a basic overgrowth, with the consequent changes in the folding of tissues leading to an abnormal pattern of differentiation. A detailed examination of the dosage effect relation for the production of the prepupal phenocopies by x-rays has been made (137). Not only the number of flies showing abnormalities, but the number of abnormalities per fly increase with dose, and the different abnormalities seem each to have a characteristic dosage-effect curve. Studies of the same sort (138) have been made for the various allels at the scute locus, with the result that bristles close to the threshold of disappearance are most sensitive to x-radiation.

The cases of homeosis have been reviewed, and a new example in *Drosophila*, the development of wing discs into halteres, has been described (139, 140).

For morphological characters, it is evident that the search for the differential reactions characterizing the special mutant effects leads eventually to cellular embryology and to the problems of the disentanglement of inter- and intracelleular reactions. The role of the cell wall as a barrier is emphasized in the comparison of the development of deficient pollen grains attached to the compensatory hyperploids: when no cell wall is formed, development is normal (141); with a cell wall between no development occurs (142).

Analysis of reaction sequences.—For the attempt to dissociate the chemical steps of the developmental processes, the study of pigment development continues to be attractive. The body color mu-

tants of *Drosophila* have now received attention (143); their study has been given a new impetus by the advances in the understanding of the process of the hardening of insect cuticle (144, 145, 146, 147). The mutants are classified according to their effects on a protein tanning and a later melanin deposition reaction. In this way the paradox that some dark body colors have light puparia is explained and the question raised of the interrelation of the two phases of pigment formation. A lower resistance (148) to toxic sprays of the mutants in *Drosophila* in which the melanic phase is suppressed suggests a change in the pore structure shown to exist in the electron microscope studies of insect cuticle (147)

The analysis of the coat colors of the guinea pig, already reviewed in these volumes, has been summarized (149). Related work on the rabbit melanins has been brought together by Daneel (150). Melanin formation is dissociated by various experimental procedures into three groups of reactions, which have been defined with respect to cytoplasmic structures. Reaction I, modified by the spotting genes, is inactivated by x-rays; the Golgi "*praesubstanz*" of Hirsch is involved. This is the precursor of the lipochondria upon whose surface dopa-oxidase is formed by reaction II; this reaction is affected by the albino allels and is the temperature sensitive reaction in the Himalayan rabbit. The amount of enzyme synthesized controls the synthesis of melanin in an aerobic reaction, poisoned by cyanide. Daneel is careful to point out what is emphasized in Wright's more extensive groups of combinations, the complexities of the reaction-net.

Similar complexities appear in the much-discussed eye pigment system of the species of *Drosophila*. The pigments, having a place in the intermediary metabolism of tryptophane, have been identified as of general occurrence in a wide group of organisms (151). Kynurenin, active as a precursor, has been identified in *Bombyx* eggs and *Drosophila* pupae of the proper genetic constitution; a substance giving the Ehrlich diazo-reaction [called by Kikkawa (152) the + chromogen] has been shown to be present in the types which should contain the "cinnabar$^+$" substance, and not in those which should lack it. A sequence of reactions is suggested by Kikkawa: tryptophane→α-oxytryptophane→kynurenine→+ chromogen→+ chrome (the so-called brown pigment). The red and the brown pigment systems are shown (153) both to be affected by the mutant claret. The formerly anomalous behavior of wild type im-

plants in claret hosts is now cleared up as being the result of the dependence of the wild type eye on the body fluid for some of its necessary kynurenin derivative. In claret body fluid, less of these substances than normal are present.

The pigment systems of the red melanophores of tropical fish are identified as carotenoids and erythropterin (154), and their distribution in the different genetic types is outlined.

Perhaps the most striking sequence of reactions reported is that concerned with the sexual reactions of the flagellate *Clamydomonas* of which a review is now available (155). The transformations of the carotenoid substances involved and correlated genetic studies are synthesized into a scheme as follows. The female or male determining genes are concerned with reactions determining the relative amounts of *cis-* and *trans-* polyene formed. The nature of the sugar to be esterified with the polyenes to form the hypothetical protocrocin is controlled by another locus; the splitting of the protocrocin to crocin is a next step, and following that the formation (under the influence of specific enzymes) of the crocetin dimethyl esters. These substances, produced by the respective gametes in definite proportions, determine their conjugation. In the female, protocrocin is also split to picrocrocin, which determines differentiation into a female gamete; in the presence of an additional specific gene, picrocrocin is split (by an enzyme shown to be present in the cells) into safranal, the determiner of differentiation into male gametes. The material is extraordinarily rich and the present summary is quite inadequate. It should be noted that reply is made in the review cited to criticisms of the work; however, so remarkable and unusually precise are some of the phenomena described that only a specialist in the field can evaluate them properly.

Sex differentiation.—The sequence of reactions in sex differentiation in *Drosophila* has been given further study with the discovery of three new intersexual strains (156, 157, 158). In *D. virilis americana*, the triploid intersexes resemble morphologically the familiar types of *D. melanogaster*, and are similarly the result of a change in balance as between X and autosomes. The two diploid types of intersex reported (*D. virilis*, *D. pseudoobscura*) are similar to each other, having the female chromosome complement, one pair of gonads, and two sets of genitalia of opposite sex. For *D. virilis*, a careful embryological study has been made. It is shown (159) that the intersex arises in development as the result not of the change

from one type of development to the other in a single imaginal disc, but as the result of the formation of a second accessory disc that develops into what female genitalia are produced. There is an active discussion (157, 158) of the role of the normal allel of these mutant genes in sex determination and of the necessary requirements for the determination of a "turning point" from one sex to the other in the development of an intersex (159).

Evidence of the XX constitution of one intersex in man, and of the XY constitution of another, is reported (160, 161). The interrelation of accessory sex glands and gonads in mammalian hermaphrodites is discussed with organs on the same side tending to be of like sex (162).

Dosage effects.—The comparative embryological analysis and the dissociation of reaction systems in mutants are strengthened considerably when it is possible to demonstrate quantitative effects on the reactions by varying the number of the genes concerned. In maize the vitamin-A content of endosperm is known to be proportional to the number of Y genes present; reexamination has confirmed the earlier work and shown in addition that the relative proportions of the different carotenoids possessing vitamin-A activity are unaffected (163).

McClintock has added to her demonstration of mutant-like effects produced in maize in homozygous deficient tissues (164).

Miscellany of physiological interest.—From the genetic point of view, the principal value of the study of mutant types lies in their use in the analysis of the nature and the function of the gene. But as has often been observed the mutant types themselves provide material for the interested physiologist, in which the internal conditions under which a given physiological process is carried out are varied. Thus, studies (165) of an anemic type affecting the embryonic cell are used in a consideration of the phylogeny of the types of blood cell. A mutant in mice with an imperforate vagina permits observation of a uterus distended by its trapped secretions (166). A hereditary "ataxia" in rabbits causing a progressive spastic paralysis is associated with a cerebellar lesion (167). A hemophilia-like condition is found in swine, autosomal instead of sex-linked as in man, but due also to a thromboplastic deficiency (168). In a new naked mutant in fowl, interesting as material for transplant experiments, although body weight and growth rate are less than normal, egg production is up to par (169). Various skeletal

mutants in fowl (170, 171) and a recessive mutant mouse with a shortened spinal column (172) have been described. Wing beat frequency in *Drosophila* has been measured stroboscopically, and characteristic frequencies found for the different species and races (173). The frequency of beat is interpreted as the resultant of wing size and thoracic muscle volume, and the complex inheritance rendered understandable in terms of the complex structural interrelations.

In plants, it is found (174) that autotetraploid *Secale* needs a seventeen hour day to set seed, although for the parental diploid a December day is adequate—a change in photoperiodism. The aborted seeds of species hybrids in *Nicotiana* are found to be due to the failure of the endosperm to grow sufficiently rapidly; the surrounding integument hypertrophies, and the differentiation of vascular tissue for the nourishment of the embryo is inhibited—a phenomenon of high interest for plant embryology (175). Following the implications of the nutritional deficiency, *Lilium* species hybrids which would normally abort have been carried through in embryo culture (176). A recessive mutant in *Triticum* is found which is much more susceptible to injury by x-radiation than normal (177); the change is not correlated with a difference in water content, heat susceptibility, or induced mutation rate.

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DEVELOPMENTAL PHYSIOLOGY

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The present review deals largely with the function of fetal organs and systems. These subjects have not been reviewed since 1940 (1, 2) and many pertinent articles have appeared in the literature since that time. On the other hand, the physiology of fertilization, mechanics of early embryo formation, chemical and physiologic aspects of organizers, polarization, and early differentiation have been very adequately reviewed recently (3, 4) and no attempt will be made to add later reports. The present review is concerned mainly with observations in fetuses of warm blooded animals, especially mammals, and with the physiologic changes which take place during birth.

Blood.—Two noteworthy articles on hematopoiesis have appeared recently (5, 6). Bloom & Bartelmez (5) studied extraordinarily well preserved human ova beginning with the thirteen day stage. Two generations of erythrocytes are sharply separated in man. Early hematopoiesis is largely confined to the yolk sac, and not until the end of the sixth week do sporadic foci appear in the entoderm. Blood formation has been well described by Bloom (7).

Possible effect on hematopoiesis of the antipernicious anemia principle remains controversial. Schlicke (8) reported numerical increase of circulating erythrocytes and decrease in their diameter and volume in newborn rats after feeding normal human gastric juice to the pregnant animals. No effect was obtained from gastric juice of patients with pernicious anemia. Jones (9) claimed some reduction of mean cell and nuclear diameters in eleven-day fetal rat blood after feeding desiccated hog stomach, but Last & Hays (10) observed no significant effect in fetuses of fifteen days when standardized potent liver preparations were used.

Some aspects of development of the blood picture in fetal life have been described in the cat (11), sheep (12), and man (13). The formation of hemoglobin proceeds at a rate which tends to maintain about fourteen volumes per cent oxygen in the blood near term (14, 15). Barcroft (14) points out a similarity between hemoglobin formation *in utero* and that in adult individuals at high

altitudes. The excess hemoglobin formed by the fetus is broken down soon after birth, providing the infant with a store of usable iron (14, 16, 17). The excess becomes available to the human infant when precautions are taken to allow the blood of the placental circuit to be transfused to the infant before clamping the umbilical cord (17, 18). That failure to delay clamping until this physiologic transfusion has occurred may lead to iron deficiency during the first year of life, has been suggested by Wilson, Windle & Alt (19). For other observations on iron metabolism, see page 74.

Total blood volume has been estimated in the maternal and fetal sheep (12, 20) and in the newborn lamb (16) and human (18, 21, 22). De Marsh, Windle & Alt (18) demonstrated a rapid adjustment of the plasma volume accompanied by hemal concentration after birth when the blood from the placental circulation was allowed to transfuse to the newborn. Blood volume formed 11.8 per cent of the body weight between birth and the third day in infants retrieving their placental blood, and only 9.6 per cent in those deprived of an average of 107 cc. of placental blood by early clamping of the cord. This difference was due to the added mass of formed elements rather than to plasma.

It was estimated that between 26 and 34 per cent of the fetal blood is in the placental circulation at the end of prenatal life (18). This is slightly more than in the sheep (12).

The proportion of nucleated red blood elements in human fetuses has been shown to diminish sharply between the seventh and twelfth weeks (23). The reticulocyte count is raised abnormally during the first postnatal week in infants deprived of placental blood at delivery (17).

The oxygen carrying power of fetal whole blood has been studied in man. Darling, Smith, Asmussen & Cohen (24) found that the oxygen dissociation curve of buffered human fetal blood at constant pH values was displaced to the left of those of the mother as well as nonpregnant women. The mean curve for maternal blood deviated only slightly from that of nonpregnant women, but more than half of the individual curves lay to the right. No significant differences between curves of premature infant's blood and those of full term infants were found. The alkali reserve of fetal blood was markedly lowered and that of the pregnant women, moderately lowered. This makes it seem probable that the difference in position of the oxygen dissociation curves would be less marked *in vivo* than *in vitro* at constant pH values. There is

little evidence from this careful study to indicate that the human fetus in the latter part of pregnancy has a different type of hemoglobin from that of the adult.

The cardiovascular system.—The old controversy regarding the course of the caval blood streams through the fetal heart has been reconsidered by several investigators and would seem to have been settled (25 to 29). There is little mixing in the fetal right atrium of the blood from the placenta with that returning from the head. The inferior vena caval stream passes through the foramen ovale to the left side and is distributed by the aorta and its great branches to the heart muscle, brain, and other rostral regions of the body. The superior caval stream passes from the right ventricle to the fetal lungs over the pulmonary vessels and to the descending part of the aorta by way of the ductus arteriosus. In an elastic model of the right atrium and its apertures Whitehead (30) demonstrated that the streams crossed when a slightly higher pressure was maintained in the inferior vena cava. The physiologic importance of the crossing blood streams is that the heart, brain, and other rostral parts of the body are able to receive well-oxygenated blood. The heart is the only organ working as hard in prenatal life as it does after birth. The brain apparently requires a high degree of oxygenation to maintain an inactive state; anoxemia of the fetal brain leads to discharge of motor centers including the respiratory center. In the fetal sheep, the pressure in the umbilical vein is very low and it is questionable if blood reaches the heart under greater pressure in the inferior vena cava than the superior vena cava (31).

A prompt rearrangement of the circulation takes place at birth when breathing starts. The ductus arteriosus is closed by activation of its highly muscular walls (28, 32). Following this immediate physiologic closure a gradual obliteration takes place as the muscle is replaced by connective tissue (23). Nerve fibers and endings in the wall of the ductus arteriosus (34) appear to be incidental to the location of the ductus. Their function has not been determined, but they do not activate the closure. Kennedy & Clark (35) could not induce closure of the ductus in the guinea pig by stimulating the vagus or other nerves. They demonstrated that the adequate stimulus for closure is oxygen which they administered via the trachea and lungs of the fetus or via the circulation directly. Inflation of the lungs with nitrogen had no effect. Thus the ductus arteriosus remains patent so long as it transmits "venous" blood in

the fetus, but constricts when the lungs begin to function at birth.

Circulation of the fetal blood is less dependent upon vasomotor and somatic motor (venopresser) mechanisms than is that of the adult. The fetal muscles are hypotonic and it is probable that the uterus aids to a limited extent in bringing about the venous return in some species. The proportion of blood in the placental circuit declines as the fetus increases in size but is still high at the end of gestation. In intrauterine asphyxiation the blood distribution is upset and the visceral organs become congested (36); the child tends to bleed into its placental vessels (37).

Barry (38) has reported that there exists a gradient along the axis of the chick heart such that the more caudal a segment lies, the more rapid is its inherent rhythmicity of contraction. Goss (39) observed the beginning of circulation of blood in rat embryos of eight mesodermal somites. The atrium acted as pacemaker and the rhythm was regular at rates of ninety to one hundred per minute. Goss (40) found that contractions begin in rat embryos of only three somites. Each heart chamber has the power of spontaneous rhythmical contraction and each has its own intrinsic rate. Contraction progresses by a wave from one end of the chamber to the other. The atrioventricular interval appears along with the atrial contraction itself. The spontaneous ventricular rhythm is inhibited by the atrium. Progressive acceleration of the heart during development of chick embryos has been studied by Alexander & Glasser (41) who call attention to the role of mechanical factors which may be involved.

Variations in the human fetal heart rate and fetal arrhythmias have been discussed by several investigators (42, 43, 44). Lund (42) believes that anoxia is the chief cause of irregular beats and slowing of the heart. Sontag & Newberry (43) report that arrhythmias, principally of sinus origin, are common and that they can be affected by external stimuli such as vibration, pressure, fetal movement, or chemical changes in the blood. They believe that immaturity of the fetal autonomic nervous system may be a factor in making these stimuli effective.

Numerous articles dealing with recording of the human fetal heart by means of the electrocardiograph and other devices have been published in the past year (45 to 51). Ward & Kennedy (52) and Geiger, Monroe & Goodyer (53) have been able to obtain positive electrocardiograms as early as the fourth and fifth months by means of special apparatus. Bernstein & Mann (54)

have succeeded in producing clear records of the fetal heart alone and have applied their technique successfully in clinical practice.

Helmholz (55) recorded carotid blood pressure optically in the newborn rat. At a body temperature of 37°C. the pressure varied between 20 to 30 mm. Hg and the pulse rate was 150 to 200 per minute. Lowering the temperature five to ten degrees decreased both pressure and pulse rate from 30 to 50 per cent. Elevation of the temperature resulted in increases in blood pressure of 10 to 30 per cent and increases in pulse rate of from 50 to 100 per cent. Venous pressures have been recorded in sheep fetuses (32).

Ingiulla (56) studied the carotid sinus mechanism in the fetal sheep through uterine incisions under a saline bath. He found that epinephrine injected into the fetal jugular vein increased the fetal heart rate and raised the fetal blood pressure. Acetylcholine had the opposite effects. Stimulation of the central end of the cut vagus of the fetus gave rise to reflex tachycardia and pressor effects. The fetal vagus was sensitive to lower thresholds of stimulation than that of the mother. Pressure changes in the fetal carotid artery gave typical sinus responses. He concluded that the fetal carotid sinus mechanism is in good functional order before birth of the lamb.

Respiration.—Fetal respiratory-like movements can occur *in utero*, especially under operative and experimental conditions. These may result in aspiration of amniotic contents. Snyder (57) has held that aspiration may be a normal physiologic process aiding in development of the lung alveoli. A tidal flow of amniotic fluid in and out of the fetal lungs is unnecessary, however, because well expanded alveoli have been observed in human fetuses with congenitally occluded respiratory passages (58). In the rat Shock (59) found that not all fetuses aspirated fluid even under adverse physiologic conditions and that excessive aspiration resulted in fetal death. Whitehead, Windle & Becker (60) proved that the guinea pig fetus at term does not normally execute respiratory movements of sufficient strength to result in aspiration of the amniotic contents. They demonstrated an initial atelectasis of the normal full term lung in which pulmonary space occupied only 2 per cent of the sectional area. When fetuses were asphyxiated *in utero* they executed rhythmical respiratory movements, aspirated fluid and the alveoli expanded until their space occupied 16 per cent of the lung. Normally born guinea pigs which had breathed for an hour had about 40 per cent space in the lungs. It is possible that the controversy over the nature of the alveolar lining can be

settled when investigators studying fetal lungs recognize that abnormal changes are wrought by aspiration induced by anoxemia accompanying intrauterine death (61, 62). Aspiration of amniotic contents to which radio opaque colloidal thorium compounds were added has been observed by German investigators (63, 64); they concluded that the intrauterine movements were not physiologic.

Initiation of respiration at birth has been discussed by the present reviewer (65) and by Barcroft (66). The respiratory function of the fetal blood has been considered on page 64. The fetus is quite adequately supplied with oxygen throughout gestation. Even though the lamb's blood was not highly saturated near term, its oxygen content per unit volume was nearly as high as in the mother, according to Barcroft (14). It was only when the oxygen in the blood leaving the fetal brain fell about 25 per cent saturation that respiratory movements began *in utero* (67). The fetal respiratory center is dormant under normal intrauterine conditions in the guinea pig but begins to discharge when anoxemia is induced; the first response observed by Windle & Becker (36) was a brief series of rapid respiratory movements followed by a slow rhythm of dyspneic gasps. Vagus and spinal respiratory reflexes are in order in the guinea pig fetus near term (36). Anoxemia attendant upon the birth process is one of the main causes of the first breath (66).

A number of articles, largely clinical, have dealt with the problem of neonatal asphyxia (68 to 73). They have been concerned especially with possible effects of anoxia upon the central nervous system and with methods of resuscitation at birth. Experimentally it has been proven by Windle & Becker (36, 74) that severe and permanent brain damage may result when anoxemia is induced in the full term guinea pig fetus and that marked alterations in behavior can occur in consequence of these changes. The efficacy of oxygen and oxygen containing carbon dioxide for resuscitating the apneic, anoxic newborn has been considered experimentally. So long as the respiratory center has not been damaged by anesthetic drugs, small amounts of carbon dioxide enhanced resuscitation (75). When anesthetic doses of pentobarbital were employed, the fetal respiratory center was rendered unresponsive to carbon dioxide, and resuscitation was accomplished more readily with oxygen alone (76).

Tolerance of anoxia has been shown by Glass & Snyder (77) to be related to degree of maturity or stage in development rather than to environment. Rabbits delivered at the onset of viability

continued to breathe for forty-one minutes in an atmosphere of nitrogen; at the end of normal gestation they breathed for thirty-one minutes; rabbits carried in the uterus three days past term[1] breathed for only seventeen minutes. Further reduction in tolerance was observed after birth until three weeks when survival time was only one and one-half minutes, the same as that of the adult.

Gastrointestinal system.—It has been determined by a roentgenographic technique that swallowing, gastrointestinal motility, absorption, and defecation *in amnio* are normal physiologic functions in the guinea pig fetus (79). Similarly in human fetuses these processes have been observed (63, 64, 80). Direct observations on cat and guinea pig fetuses (81, 82) revealed that the earliest activity was simply a localized contraction in the gastrointestinal tract of seventeen-millimeter embryos. Spontaneous gastric and intestinal peristalsis was present in twenty-seven millimeter cat fetuses and thirty-five millimeter guinea pig fetuses. Peristalsis was observed earlier than rhythmic segmentation in the cat, but the converse was true in the guinea pig. Peristalsis became progressively more active up to fifty days of gestation (term about sixty-seven days) and thereafter became less active in the small intestine. The stomach, cecum, and colon of the guinea pig fetuses were highly motile at all stages. Anoxia suppressed peristalsis which gave way to generalized rhythmic segmentation and ultimately to less of tonus and to pendulous writhing movements. Anoxia initially excited the inactive intestines during late fetal life. In man the earliest time at which passage of fluid into the esophagus has been observed radiographically is about nine weeks (80).

Nervous system.—Functional aspects of the brain in fetal life and soon after birth have been considered by several investigators. Riese (83) had the unusual opportunity of studying activity of bear cubs shortly after birth and examined the structure of their brains. He declared that the nervous system of the newly born is embryonic and corresponds to that of a four months old human fetus. He found that the midbrain was the highest level containing matured nervous elements. No evidence of the higher nervous functions was apparent at birth. Kennard & McCulloch (84) explored the cerebral cortex of a three weeks old monkey electrically. Area four was the only region from which responses were elicitable. These were contralateral and were less discrete and of slower onset

[1] For a discussion of the method of carrying fetuses *in utero* past term, see Coco (78).

than in the adult. They were easiest to elicit from the face region, the hand area was next, and no responses were obtained from the leg. Relatively high voltages were required.

The cholinesterase concentration of different portions of the nervous and muscular systems has been estimated by Nachmansohn (85) in the fetal sheep and Youngstrom (86) in human fetuses. It was observed that the enzyme occurred in greater concentration than in adult tissues. It reached a high level in the spinal cord first, the brain stem next, and the cerebral cortex last. In striated muscle the enzyme was highly concentrated at the regions of nerve endings at an early age, long before end plates appeared. The increasing concentration of cholinesterase in the nervous system parallels functional development.

Early responsiveness of the nervous system of the human fetus has been studied by Fitzgerald & Windle (87). They were able to elicit spinal type reflexes about the eighth week of gestation in specimens twenty to twenty-six millimeters long at the operating table before asphyxiation of the fetuses. During progressive anoxia stronger mechanical stimulation was required and the responses became generalized. These and other recent studies indicate that the cerebrum is late in reaching a functional state. Spinal and lower brain stem levels contain the first neurons which can be induced to discharge and the fetus is essentially a bulbospinal individual.

Hogg (88) correlated development of sensory nerve endings with responsiveness of human fetuses of eight to fourteen weeks of gestation to cutaneous stimulation. Endings develop late, and he suggested that the early excitation of the sensory nerves depended on deformation of growing tips of fibers which were displaced by pressure on surrounding tissues.

The question of genesis of behavior from a "total pattern" or as a progressive integration of more or less discrete functional components has received recent attention. One attempt (89) to establish an anatomical basis for the former theory finds no confirmation in experiments of Rhines & Windle (90) who have observed that the fundamental structural plan of the embryonic mammalian brain can be correlated with the observations of local reflex movements. It was proved experimentally by Rhines (91) that descending tracts do not reach the motor centers of the medulla oblongata and spinal cord of chick embryos until after the first somatic movements can be observed.

The relation of anoxia to early function of the nervous system in human embryos has been mentioned (87). In the cat Windle & Becker (92) found that fetal irritability was first enhanced by progressive anoxemia. Simple reflexes of the limbs were soon abolished, however, and the head and trunk movements became less discrete, more tonic, and more massive. Partial anoxemia induced rhythmicity of discharge of certain neurons, especially those of the respiratory center in cat fetuses of twenty-eight millimeters.

Reflexes obtained in cat fetuses from stimulating regions supplied by the three divisions of the trigeminal nerve exhibited interesting reversals (93). For example the head moved contralaterally at twenty-five days, reversing to homolateral at twenty-seven days, and again flexing contralaterally at thirty days when one maxillary region was stimulated and while the placental circulation was intact. It was suggested that these reversals came about in consequence of a fluctuating preponderance of commissural or associational interneuron connections in the developing brain. During anoxia similar reversals occurred at earlier stages in development, anticipating, as it were, the changes about to take place under better physiologic conditions. Because reversals during anoxia were new patterns not previously encountered during preanoxic conditions but about to manifest themselves under such conditions, a recapitulation of the development of reflexes by anoxial destruction of inhibitory centers seemed unlikely.

Special emphasis has been placed on the danger of misinterpreting the results of experiments on the fetal nervous system when the effects of anoxia are not given proper consideration. This was shown by Becker (94) in the incubating chick, where coating the shell membrane with vaseline to render it transparent led to early anoxial death of the embryos.

The brain of the fetus and newborn is less susceptible to permanent damage by anoxia than that of the adult (74). Survival times are prolonged and gasping outlasts all other reflexes (77, 95, 96, 97). Species differences have been observed, the immaturely born rat having the longest survival time and the relatively mature new-born guinea pig, the shortest (77, 96). The tolerance of young rats and dogs to anoxia and hypoglycemia is due to several factors including low cerebral metabolic rate, poikilothermia, and the presence of some anaerobic source of energy (96). Himwich and others (97) have shown that the prolonged survival time of infant rats, despite inhibition of the cytochrome oxidase system with

sodium cyanide, demonstrates the function of the latter factor. The rapid death after injection of iodoacetic acid and exposure to an atmosphere of nitrogen suggested anaerobic conversion of carbohydrate to lactic acid as the source of this energy. An increase in carbohydrate stores permits longer exposure to nitrogen and a decrease has the opposite effect (98, 99). Oxygen consumption is lower in the brain of the young dog than it is in the adult. With growth there is a progressive increase in oxygen consumption from the medulla oblongata rostrad (100). The cerebrum of young animals is less susceptible to effects of alcohol and pentobarbital than that of adult animals (101).

Tibet, Fazekas & Himwich (102) have studied action potentials in young kittens. They observed a gradual development of frequency, regularity, and amplitude of cortical electrograms during infancy. Activity was induced in comparatively silent brains of the young by metrazol or strychnine as well as by acoustic stimuli. Responses differed from those of the adult and higher doses of metrazol were required.

Kellogg (103) has described a method of recorded human fetal activity from the maternal abdomen. He observed that the rostral portions of the fetus were more active than the legs at first, but that the legs became increasingly active in later stages of development. Newberry (104) reported a great variation in amount of human fetal activity, some individuals being active 75 per cent of the time and others only 5 per cent in late gestation. Sharp kicking movements became more frequently apparent and slow squirming less prevalent from the sixth month onward. Rhythmical movements and "hiccups" were infrequent.

Flexner, Flexner & Straus (105) reported that two critical periods, the first about half way and the second about four-fifths of the way through gestation, were found in the morphological differentiation of the fetal cerebral cortex. Both were characterized by rapid increase in size of the nerve cells, by changes in their form and by abrupt changes in the quantity or pattern of the Nissl substance in the cytoplasm. During the first period, cytochrome-cytochrome oxidase activity showed a distinct rise and during the second period, the Q_{O_2} increased to the level characteristic of the adult. Variations in cytochrome-cytochrome oxidase activity at different parts of the gestation period were not reflected in corresponding variations of Q_{O_2}. Cytochrome oxidase activity was found constant at all stages of fetal development investigated. The

increase in cytochrome-cytochrome oxidase activity with increasing fetal age consequently appeared due to an increase in concentration of cytochrome-*c*. The $Q_{O_2}^{CN}$ of the fetal and adult cortex was about one.

Tyler & van Harreveld (106) studied respiration of the developing rat brain at different ages after birth. During the first week they found the lowest oxygen and glucose utilization but the highest glycolytic activity. The highest oxygen and glucose utilization occurred between the fourth and seventh weeks with a decrease in glycolytic activity. The adult, and somewhat lower, level was reached about the twentieth week. At birth the medulla oblongata had a higher respiration than the pallium, and in the adult the order was reversed. Racker (107) obtained lower values for cerebral cortex than for medulla oblongata of the human fetus of five to six months.

Placental transmission.—Many aspects of placental function, especially its role in nutrition of the fetus, have been reviewed recently by Huggett (108). The question of control of placental function has been discussed in another article by the same author (109) who points out that the primate placenta changes its function from a secreto-storage mechanism to a diffusion mechanism by loss of tissue by autolysis. The survival of the embryo depends in part on the extent of the diffusion membrane which in turn depends on placental weight. Placental development and degradation seems to be controlled independently of the fetus and of the normal maternal metabolism. This power of development of the placenta enables it to compete successfully with the fetus and still more with the mother for food supplies when these are limited.

Flexner, Pohl & Gellhorn (110 to 117) have studied the transfer rate of radioactive sodium from mother to fetus in a series of species having epitheliochorial (sow), syndesmochorial (goat), endotheliochorial (cat), and hemochorial (rabbit, rat, and guinea pig) placentas. Progressive increase in permeability with advancement in pregnancy was correlated with histological changes such as thinning of the epithelia. The types of placentas appearing morphologically less permeable were found to be in fact less permeable per unit weight to the transfer of radioactive sodium. At comparable stages in pregnancy the placenta of the rat was two-hundred times more permeable than that of the sow. The transfer rate appeared to parallel the relative growth rate of the fetus in each species.

Using heavy water as a tracer material Gellhorn & Flexner (118) found a ninefold increase in the rate of transfer of water across the guinea pig placenta between the twenty-eighth day and term and could correlate this with changing placental morphology. When the fetus was growing with greatest rapidity, the rate of supply of water to a unit weight of fetal tissue was greatest. Water was found to traverse the placenta with great speed. A volume of water equal to about twice the fetal weight passed from mother to fetus in one hour. A complete turnover of the water of the amniotic fluid occurred about once an hour.

Nielson (119) reported the passage of radioactive phosphorous in the form of a phospholipid molecule to be a very slow process in the rat. It was thought probable that phosphorus passed the placental membranes in a smaller molecular form and was synthesized by the fetus into phospholipid.

Pommerenke, Hahn, Bale & Balfour (120) recovered radioactive iron from human fetal blood forty minutes after feeding it to the mother. This speed of transfer suggests that plasma rather than the red corpuscles is the vehicle. The distribution of radioactive iron in fetal tissues showed a wide dissemination of the isotrope, with greatest concentration in the red corpuscles and liver.

Investigations of Bodansky & Duff (121) have dealt with parathyroid function and the storage of calcium and phosphorus. They found that diets nearly devoid of these elements did not necessarily prevent normal fetal growth and storage of calcium and phosphorus when the maternal parathryoid glands were intact. However lack of parathyroid secretion during pregnancy disturbed mineral metabolism on both sides of the placenta. Disturbances of the calcium-phosphorus ratio by an overabundance of calcium in the diet of rats with intact parathyroids led to impairment of the deposition of bone salts in the fetuses. It was concluded that development of the fetus and its ability to store calcium and phosphorus depend on maintenance of suitable concentrations of these elements in the maternal and presumably in the fetal blood streams.

Other investigations and discussions of placental permeability have been concerned with passage of vitamins (122 to 126), antibodies and products of metabolism (127, 128), the sulfonamide compounds (129, 130, 131) and other drugs (132, 133, 134, 135) across the placental membranes.

Changes in the content of iodine compounds of the thyroid

during gestation were studied by Rankin (136) who found inorganic iodine in pig fetuses six to eight centimeters long. Thyroxine and diiodotyrosine were detected at eight to nine centimeters (fifty-two days) and the thyroid follicles and colloid appeared.

Lelkes (137) observed that epinephrine is formed by the human adrenal medulla in the third month. It is found in the paraganglia in the fifth month of gestation.

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PHYSIOLOGY OF BONE

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This review summarizes the advances in the physiology of bone since a review by Huggins (1), or during a period of five to six years. During this period other reviews have appeared on some aspects of the physiology of bone (2), mineral metabolism (3), the pathology of rickets (4), vitamins and sterols (5 to 9), hormones (10, 11), chemistry of calcification (12, 13, 14), phosphorus (15), and fluorine (16). The review of Schmidt & Greenberg (17), supplemented by the later review of Greenberg (18), is still a standard source of information concerning the behavior of calcium, magnesium, and phosphorus in the animal organism, as is that of Bills (19) for the sterols, including vitamin D. In so far as is possible, duplication of the material covered by these reviews will be avoided, and reference made to them for their contributions to the subject of this review. The Cumulative Index to Volumes I to X of the *Annual Review of Biochemistry* is a rich source for references to many aspects of the physiology of bone and cognate subjects. Leriche's lectures on the physiology and pathology of bone (20), supplementing the earlier monograph of Leriche & Policard (21), have appeared during the period under consideration, and both of these books present original and refreshing points of view.

This review purports to cover systematically, albeit incompletely, only the physiology of bone; reference will be made to other supporting tissues, notably teeth and connective tissue, as occasion arises in the discussion of bone. The pathology of bone will be referred to only incidentally to illustrate various aspects of pathological physiology. The treatment throughout this review is of bone as a tissue, with little reference to the functions of the skeleton as a whole, or to the shape, adaption, or development of individual bones.

Growth and development of bone.—The general problem of growth has been reviewed, with considerable attention to the growth of bone (22). Recent work on the development of bone, both as a tissue and as an organ, has also been reviewed (2).

Heinen (23) has implanted outgrowths from tissue cultures of bone to the anterior chamber of the eyes of rats. The grafts became vascularized and a chondroosseous tissue developed. Primitive bone marrow then appeared, and there was invasion and replacement of the earlier tissue by true bone. Finally, a disc-shaped bone, adapted to the size and shape of the anterior chamber, developed with central fatty marrow, and further growth did not occur (24). In rats on adequate diets the new bone calcified normally; in rats on diets low in phosphorus and vitamin D the intraocular bone was rachitic.

The problem of calcification in developing and growing bones, and the relations of hormones and vitamins to the growth of bone, are reviewed below.

Regeneration of bone.—Most of the literature on regeneration of bone is concerned with the healing of fractures and is reviewed below. By an ingenious use of transparent chambers in the ears of living rabbits (25), the regeneration of cartilage and the development of bone were observed *in vivo*. When bone appeared, it arose spontaneously in the midst of new nonvascular cartilage and was later invaded by capillaries. Regeneration and reorganization of the epiphyses of the long bones, including the epiphyseal cartilage plates, have been studied in rats (26).

Ageing of bone.—The problems of ageing of the skeletal system have been extensively reviewed (27), and with special reference to hormones (28). During the life span of normal mice (29) the skeletal tissues undergo changes which may be divided into three periods, with great differences in the rate of skeletal ageing in different strains of mice.

Physiological unity of the osseous system.—In an interesting series of papers Roche and his collaborators (30, 31, 32, 33) have developed a concept of the physiological unity of the osseous system, basing this concept on the sequence of events in the skeleton as a whole following a fracture of one bone, and especially increase in the phosphatase activity and demineralization which affect the fractured and intact bones alike. A study of the metabolism of the rat following fracture of the femur (34) has led to the conclusion that there is a generalized increase in catabolism. The denervated bones of rats and cats (35) remain normal for long periods; bones and joints are evidently not supplied with nerves having a specific trophic function.

Histophysiology of Bone

Histogenesis of bone.—Because of the rapidity of the changes in medullary bone in birds during the egg-laying cycle, such material, which offers unusual opportunities for the study of bone-forming potentialities of recticular cells and of the origin and fate of cells associated with the formation and destruction of bone, has been utilized for this purpose (36). It is concluded that in the pre-ovulatory bone-forming period, reticular cells become osteoblasts, and these in turn become osteocytes. In the bone-destroying phase after ovulation of the first egg, osteoblasts and liberated osteocytes become osteoclasts. The latter may turn into osteoblasts during resumption of bone formation. In the post-ovulation period, osteoblasts and osteoclasts again become reticular cells. In the embryonic rat, formation of osteoblasts is described (37) from vacuolated cartilage cells and from the ectoperiosteum. The continuation of cartilage cell dedifferentiation is questionable. Osteoclasts arise from differentiated cells of the bone marrow and from other cells originating in the destroyed tissue (38).

Tissue cultures of bone.—To the experiments of Fell and others, using material from chick embryos, which have been reviewed by Newton (2), have been added those of Heinen (23), who cultured the tibias of young rats and observed formation of cartilage and bone, with calcification of the latter. Conditions for the culturing of tibias from chick embryos have been described (39), and the role of tension and pressure in chondrogenesis *in vitro*, on chick bone rudiments, has been studied (40).

Heterotopic bone.—Huggins reviews the earlier work on production of heterotopic bone (1). Of great interest is the more recent work of Levander (41, 42, 43) and of Annersten (44, 45) from which it is concluded that specific chemical substances in bones are capable of eliciting osteogenic potencies in undifferentiated mesenchymal tissue. Their conclusions are based in part upon experiments in which formation of bone was induced in soft tissues by the injection of alcoholic extracts of bone; these results, however, have not been confirmed by others (46). Formation of bone in the human eye is described (47, 48).

Calcification in developing and growing bone.—Calcification in the bones of embryonic and young animals has been studied by a method which avoids decalcification and affords a positive stain

for the bone salt (49). In most bones of the embryonic rat (50) the periosteal bone is laid down and there is extensive calcification in the cartilage model before there is any penetration by blood vessels. In embryonic rats and in young rats, puppies, and kittens, calcifiability is conferred upon cartilage matrix as the adjoining cells become hypertrophic, and upon bone matrix as it is laid down. The concept of "physiological osteoid" or of uncalcified osseous tissue, in the sense of a necessary stage in the formation of bone, is not valid, but local areas of osteoid borders on calcified bone, attributed to failure in the local supply of bone minerals, may occur under physiological conditions. In the puppy the occurrence of such borders is greatly increased when vitamin D is omitted from a diet adequate in other respects (51).

Resorption of bone.—It is generally assumed, although not proved, that osteoclasts are in some way responsible for the resorption of bone. From this to the assumption that they resorb bones by phagocytosing it is but a short step, but there is little evidence for such a mechanism. To those who adhere to the belief that bone is decalcified under the influence of the parathyroid hormone, by an increase in the solubility of the bone salt in the circulating fluids of the body (11), resorption of the organic matrix of bone is a reaction to decalcification.

Evidence on these points has been accumulated under a variety of conditions, under all of which it appears that the body has access to the calcium salts in bone only by destruction of the bone itself, including the matrix (36, 49, 52, 53). It thus appears that decalcification of bone is a misnomer, and that the concept of destruction of bone by resorption, involving simultaneous dissolution of both its organic and inorganic constituents, must be substituted for that of decalcification. What is frequently called decalcification of bone (54) is probably failure of calcification of new osteoid tissue formed in the growth or physiological turnover of bone.

Nor has any evidence that resorption of bone is accomplished by phagocytosis by osteoclasts appeared. On the contrary, under conditions leading to extensive phagocytosis of the bone salt and of necrotic debris by macrophages and other cellular elements of the marrow, the osteoclasts remain singularly free from this material (53).

Chemistry of Calcification

State of calcium in the fluids of the body.—The finding (55) that the calcium ion concentration in the fluids of the body is determined chiefly by the total calcium and total protein concentrations and by the ionization constant of calcium proteinate, has received additional confirmation (56 to 62). The most convincing evidences of the validity of the fundamental calcium-protein relationship has been obtained by an ingenious use of the ultracentrifuge (63) by which the dissociation constant of calcium proteinate has been determined with a high degree of accuracy. Contrary to the findings of many investigators it was found that, over a range of pH 6.3 to 8.5, the calcium-combining power of casein remains unchanged, although with serum a rise in pH caused a fall in the concentration of diffusible calcium when examined by the same method (64).

On the other hand, the findings by use of the ultracentrifuge (64, 65) appear to cast doubt on the accuracy of calcium ion concentrations as estimated by application of a single mass-law equation to values for total calcium and total protein from human serum. Others have questioned the validity of the approximation for serum (66, 67), with evidence based upon the findings in the blood of herbivorous animals in which there may be considerable amounts of citrate (55). A final decision as to whether a diffusible, nonionized form of calcium is present in human plasma in serum, under any conditions commonly met with, and in amounts sufficient to invalidate the McLean-Hastings formulation, must await more accurate methods for the direct determination of calcium ion concentrations.

Composition of bone salt.—There are excellent reviews of the literature concerning the composition and crystal structure of the mineral constituents of bone and teeth (12, 13, 14). There is now virtual unanimity to the effect that the fundamental crystal structure of the bone salt is that of an apatite (13), of which the prototype is fluorapatite, $Ca_{10}F_2(PO_4)_6$. It has, however, been established that an earlier conception of the bone salt, which postulated replacement of F_2 by CO_3, is untenable (68), and that the essential substitution is that of $(OH)_2$ for F_2, giving hydroxyapatite. It is well known also that other substitutions take place in the molecule, the most common being magnesium, sodium, and

potassium for calcuim, that the bone salt is not a distinct chemical individual, of constant composition, and that changes and substitutions continue to occur long after the mineral is deposited (12). In fact, it is denied that hydroxyapatite can exist in an aqueous system as a unique, stoichometric compound (13), as there is in the ternary system, a continuous series of solid solutions having an apatite lattice. There remain, however, some differences, partly in point of view, with reference to the further details of the structure of the bone salt. In order to account for the carbon in the crystal, now excluded from the fluorine positions, evidence has been brought forward for its substitution in certain of the calcium and phosphorus positions in the apatite lattice (14); substitution of carbon for calcium, however, has more recently been denied (69), and a different type of substitution for phosphorus proposed. In the latter view, the basic structure of the bone salt is that of a hydrated tricalcium phosphate, a deviation from the conception of hydroxyapatite. Finally, other workers (70, 71) have returned essentially to an earlier view of the chemical composition of bone, which is that carbonate is not a part of the apatite molecule at all but represents some sort of an admixture with it.

Solubility of bone salt.—From the above the difficulties inherent in attempting to express the solubility of bone salt in terms of a solubility product are apparent. The ion product $[Ca^{++}]^3 \times [PO_4^{---}]^2$ is defended (12), and the errors in earlier work are attributed to adsorption of calcium ion, phosphate ion, and perhaps other ions onto the solid phase. On the other hand, it is contended (72) that instead of decreasing with the amount of solid phase (73) this ion product increases as more solid is present and varies also with the pH of the solution. The contention (74) that "the product $[Ca^{++}]^3 \times [PO_4^{---}]^2$ implies a fifth order reaction" is, however, incorrect, for the reason that a solubility product constant, being based on thermodynamic considerations, is independent of the pathway followed by the reactions. More difficult to meet, however, is the statement (13) that the "concept of solubility product is meaningless without reference to a known solid phase of comparatively simple ionic composition."

To avoid some of these difficulties and to account for some of the peculiarities in behavior of the systems examined, it is proposed (74) that tricalcium phosphate be regarded as $Ca(CaPO_4)_2$, and that the ion product $[Ca^{++}] \times [CaPO_4^-]^2$ be used for comparison

of solubilities under varying conditions. The evidence for formation of $CaHPO_4$ as an intermediate step in the deposition of bone salt has been reviewed (12), and evidence is presented (75) for the presence of this compound in solution in undissociated form.

Biological solubility.—Much of the difficulty in defining the solubility of the bone salt in terms of a solubility product, is that equilibrium between the solution and the solid phase may not be attained, even after shaking for days (72). To avoid the necessity of equilibrium, the concept of "biological solubility" with the use of a biological endpoint has been introduced (76). The limit of biological solubility is the point at which deposition of the bone salt just occurs in the cartilage matrix of rachitic cartilage, either *in vivo* or *in vitro*, indicating that the solution from which the salt is deposited is saturated with respect to it. The method, of course, is simply a refinement of one long in use.

It has been found (76) that the local calcification mechanism in rachitic cartilage is active in solutions varying in hydrogen ion concentration over a range of at least pH 5.7 to 8.7, a remarkable range for a biological phenomenon. By using this entire range it is possible to obtain a maximum separation of the curves for the various ion products in solutions containing calcium and phosphate. At pH 7.0 and higher, the critical point for calcification is correlated closely with the solubility product $[Ca^{++}] \times [HPO_4^{=}]$, as found by Shear and his associates (77). On the acid side of pH 7.0, however, the correlation is with the product $[Ca^{++}]^3 \times [PO_4^{---}]^2$ of Logan & Taylor (73). The curves for the two products cross at approximately pH 7.0, and the generalization may be made that both products must be exceeded for calcification to occur. Since the product $[Ca^{++}] \times [HPO_4^{=}]$ is higher at pH 7.4, it appears to be critical for calcification *in vivo*, as proposed by Shear and his associates (77). Similar findings *in vivo* have been reported (51).

Whatever the interpretation of these findings, which are offered with all due reservations in advance of submission of the complete data, they appear to be adequate for an expression of the humoral conditions under which calcification will occur and under which it will fail in the growing animal. They do not account for the fact that adults, with concentrations of calcium and phosphate in the plasma identical with those of the rachitic child, can still carry on the physiological turnover of bone mineral and can calcify the callus of a healing fracture (78).

Phosphatase.—The importance of phosphatase in calcification, vigorously supported by Robison (79), still remains to be demonstrated. Its role in calcification and its behavior under various physiological and pathological conditions are reviewed (80). The influence of various hormones on the phosphatase content of the bones of rats has been described (81, 82). In accord with a theory previously advanced (83) it has been shown (51) that phosphatase is associated with the layer of osteoblasts applied to the surface of growing bone and not found in the matrix of osteoid tissue awaiting calcification.

The local factor in calcification.—Robison (79) left the problem of the "second mechanism," otherwise known as the "local factor," in an unsatisfactory state, but clearly felt that it was enzymatic in nature. McLean and others (84) have again approached this problem, also with the working hypothesis of an enzymatic mechanism. This point of view was supported by the observation that heating to 60°C. causes a complete and irreversible inhibition of calcification of rachitic cartilage *in vitro*. Other influences, including iodoacetate, fluorine, alcohol, acetone, chloroform, drying over phosphorus pentoxide, and leaching in phosphorus-free saline solutions, cause a partial inhibition, usually easily overcome by adding an excess of phosphate to the solutions used. A search for a method of producing a satisfactory reversible inhibition of the local factor, with which the nature of the process could be further investigated, has so far failed.

It has been reported (85) that there is a correlation between the appearance of glycogen in the cartilage cells and the calcifiability of the matrix. Efforts to show that the calcification is dependent upon anaerobic glycolysis in the tissue have failed (84). Glycolysis may be inhibited, to the extent of 70 to 90 per cent, by iodoacetate or fluoride, and this inhibition is accompanied by a slight reduction in calcification, but the latter is easily overcome by adding slightly more phosphate. Moreover, glycolysis, in the absence of inhibiting agents, proceeded at the same rate, whether or not the incubating medium contained enough phosphate to induce calcification in the cartilage matrix. Gutman & Gutman (86) have demonstrated the presence of a phosphorylase in calcifying cartilage and again suggest a role of the glycogenolytic cycle in providing inorganic phosphate for calcification of the cartilage matrix.

Addition of adenosinetriphosphate and of creatine to the incu-

bating medium had no effect upon calcification. Addition of glutathione and thioglycolic acid, both of which are reported to inhibit phosphatase activity, still gives excellent calcification in the presence of a phosphate ester and in the absence of inorganic phosphate (84).

There have been recurring suggestions that the local factor is the result of an increase in pH in the region where deposition of the bone salt is to occur. This idea, dismissed by Robison for the lack of experimental support (79), continues to find some favor but no proof (87).

Endocrine Effects

Anterior pituitary.—Despite some earlier doubts (88) as to the existence of a specific growth promoting hormone in the anterior pituitary, its existence is now generally accepted (10). The effects of hypophysectomy on the epiphyseal growth of bone, not in themselves necessarily wholly dependent upon absence of the growth hormone, have been described (89 to 95). Under all conditions except those in which epiphyseal union had occured, administration of the growth hormone obtained from alkaline extracts of the anterior pituitary resulted in resumption of normal growth, and under certain conditions a return to a more "youthful" type of epiphyseal growth zone occurs (90). The effects of the growth hormone upon epiphyseal growth, when administered to unoperated animals, have also been described (90, 92, 93, 96), and it has been shown (97) that the hormone, administered to rats during the period of weight plateau at the age of six months or shortly thereafter, induces histologically recognizable evidences of active growth in quiescent or lapsed epiphyseal cartilage plates and the adjacent spongiosa. There is agreement (10, 97) that the effects of the growth hormone are exhibited upon bone as well as upon cartilage, and that the designation of the growth hormone as "chondrotrophic" (89) is too narrow. Silberberg & Silberberg (96) have reviewed their series of papers upon the effects of an acid extract of bovine anterior pituitary under a variety of conditions, and have compared these effects with those produced by the conventional preparations of the growth hormone.

In addition to the growth hormone, the anterior pituitary yields gonadotrophic hormones (88, 98) and others are derived from chorionic tissue. Because the effects of these hormones upon bone

are indirect and depend upon stimulation of the production of hormones by gonadal tissues, they are reviewed together with the corresponding sex hormones.

Thyroid.—The growth promoting activity of the thyroid is believed (99) to be a phase of its action on metabolic processes in general. Conflicting statements are made concerning a possible synergistic action of thyroid and the growth hormone of the anterior pituitary (100, 101, 102). The histological changes in the skeletal tissues of immature guinea pigs following thyroidectomy (103), those in adult male rats thyroidectomized when one month of age (104) and those in mice following administration of thyroxin (105), have been described.

The occurrence of osteoporosis in clinical hyperthyroidism continues to be reported (106, 107). Rats fed sufficient thyroid substance to produce severe hyperthyroidism failed to show decalcification or failure of calcification of their bones (108, 109), but premature cessation or marked retardation of growth at the epiphyseal junctions did occur in young rats. Changes in the epiphyseal cartilages of children, characteristic of hypothyroidism, are reported (110, 111, 112).

Thymus.—The phenomenon of acceleration of growth and development in successive generations of rats treated with thymus extract, reported by Rowntree and co-workers (113), has failed to find confirmation from the experiments of later workers (114, 115). Likewise, thymectomy, in six successive generations, failed to influence growth or development (116). The discrepancies appear to be only partly explained by differences in diet (115).

Sex hormones.—An extraordinarily interesting phenomenon, first described by Kyes & Potter in 1934 (117), has been exploited during the period covered by this review. This is the development of a secondary system of bone in the marrow spaces of the long bones of birds, now commonly referred to as "medullary bone," and serving the function of storage of calcium to be required for the egg shell. The phenomenon is apparently common to all birds, but its cyclic characteristics are best studied in such birds as the pigeon, in which the egg-laying cycle is brief. The details of the transformation in the pigeon during the normal egg-laying cycle have been described (36) and are reviewed above.

There is a considerable body of literature on the production of this medullary bone by the administration of estrogens to chickens

(118, 119), pigeons (120), ducks (121), and sparrows (122). It has also been produced in sparrows (123) and in baby chicks (124) by the administration of gonadotrophic hormones to birds with intact ovaries. For the pigeon, it has been shown that the ability of male birds to produce medullary bone, under the influence of administration of estrogens, is seasonal, and that the seasonal effect apparently depends upon the fact that androgen, as well as estrogen, is necessary in this species (125). In the sparrow (122) a similar synergistic effect has not been found.

In the female bird, it would appear that the development of medullary bone is mediated by the action of estrogens, the role of androgens, in the female, being still uncertain. In this respect the formation of medullary bone is similar to other phenomena of the egg-laying cycle, notably increase in the fat and phospholipid content of the plasma, appearance of a phosphoprotein, serum vitellin, in the plasma (126, 127), and a pronounced hypercalcemia (128, 129). All of these phenomena begin, in the pigeon, with activation of the ovary by the act of copulation and occur *pari passu* with the maturation of the ovarian follicle. That the mechanisms are not entirely explained by the action of estrogens is indicated by the fact that experimentally induced growth of medullary bone is neither as rapid nor as extensive as in the normal cycle in the female, and by the fact that hypercalcemia and medullary bone can be produced together or independently in the pigeon, according to the experimental conditions (125).

During calcification of the egg shell the process in the bone marrow is rapidly and sharply reversed, with extensive and rapid dissolution of the medullary bone, accompanied by large numbers of osteoclasts (36). The mechanism by which this reversal is mediated, although strongly suggestive of hormonal influences, remains unexplained. There is no evidence that the parathyroid hormone is concerned (125). A similar dissolution of medullary bone occurs with termination of the egg-laying cycle, when the ovary returns to the resting condition.

The bearing of these observations on birds upon the relationship of sex hormones to the bones of mammals has not been wholly clarified, and there are apparently species differences. In the mouse medullary bone of endosteal origin, following administration of estrogen and leading to virtual obliteration of the medullary cavities, has been described (130, 131). The breaking strength

of femurs so treated is greatly increased (132). The effects of estrogens on the skeleton of the mouse (including both pubic resorption and formation of medullary bone) are inhibited by simultaneous administration of androgens (133). In the rat and guinea pig a condition superficially resembling that in birds occurs, but the increase in spongy bone is chiefly, if not all, in the region of the epiphysis and is due to a failure of resorption of spongy bone during the growth in length of the long bones (134, 135, 136).

The effects of ovariectomy (137) and of progesterone (138) on the skeletal tissues of immature guinea pigs have also been studied. A form of osteoporosis, occuring in women after the menopause and responding favorably to therapy with estrogens, is attributed to the postmenopausal state (139). Attempts to influence the healing of fractures (140, 141) by administration of estrogens have not yet demonstrated a consistent beneficial effect. The balance between estrogens and androgens may maintain normal control of the shape of the long bones (142); osteitis fibrosis cystica may be due to a long-continued excess of estrogen (143). Estrone and diethylstilbestrol inhibit epiphyseal growth of bone in rats and mice (144); the effect, which is not counteracted by the growth hormone, is due to a local action.

Androgens and gonadotrophins.—The long bones of rats castrated at the age of twenty-one days and killed when one year of age are significantly shorter than those of control rats (145). Small doses of testosterone stimulate the growth of castrate rats (146), and there are seasonal differences in the response to testosterone (147). Large doses of testosterone depress the growth of rats (148) or have no effect (149, 150). Testosterone inhibits for some time absorption of cartilage and metaphyseal bone in mice, but to a lesser extent and for a shorter period than does estrogen (151). In man, the influence of the gonadotrophic hormone appears to be correlated with the growth that occurs normally at the time of puberty (152): chorionic gonadotrophin and male sex hormone both cause rapid ossification and ageing of epiphyses, and this effect is counterbalanced by a rapid increase in length of the bones. Best results with male sex hormones and chorionic gonadotrophins were obtained in cases with combination of growth deficiency and genital retardation (153). The effects of chorionic gonadotrophin are probably the result of the production of male sex hormone (154).

Parathyroids.—Little new has been added to previous knowl-

edge concerning the mode of action of the parathyroids on bone. Whether bone gives up its mineral to the blood as the result of a direct action of the hormone on the cellular elements of bone, or whether the action is indirect by way of the kidney or otherwise, remains unresolved. One of the originators of the first school of thought has transferred to the second (155), basing his defection upon his failure, in common with that of others, to induce hypercalcemia in nephrectomized animals by administration of parathyroid extract, while one of the originators of the second school, who still adheres to it, recognizes a direct effect of the parathyroid upon bone, in extreme cases, by postulating a form of "parathyroid poisoning" or "hyper hyperparathyroidism" (11). Previous reports that parathyroid extract, when administered to dogs, leads to a rapid increase in the urinary excretion of phosphate and (usually) to a decrease in the plasma phosphate level, have been confirmed (156). Attempts to relate these phenomena to the renal clearance have given both positive (157) and negative (158) results. On the other hand, evidence has been cited (53) in favor of the view that the parathyroids exert a direct effect upon bone.

It has been shown that the hypertrophy of the parathyroids in experimental rickets occurs only (159) or predominantly (160) in rickets associated with deprivation of calcium, rather than in that associated with a low intake of phosphorus, and also that the development of hypertrophy is correlated with a low blood calcium level (159). The now well-recognized secondary hypertrophy of the parathyroids in chronic renal disease, leading to extensive bone changes (renal rickets) and to metastatic calcification, is also attributed to a fall in serum calcium, resulting from hyperphosphatemia (161). The osseous findings in chronic renal insufficiency in adults, in most cases, have been attributed to acidosis, rather than to secondary hyperparathyroidism (162).

Adrenals.—Ablation of the adrenals leads to a failure of endochondral bone formation similar to that which follows hypophysectomy (95); it has not been shown that this effect is specific.

Storage of Calcium

It is shown in this review that in the bird, where unusual demands are made upon stored calcium, a special mechanism protects the skeletal system. In mammals, however, the function of storage is carried on by ordinary skeletal bone, predominantly

in the spongy bone near the epiphyses of the long bones. From there it is liberated by resorption of small amounts of bone, under the mediation of the parathyroid hormone. There is no evidence that the latter influences the deposition of calcium.

The concept of an "optimum," as opposed to an "adequate," intake of calcium has been emphasized (163); when the intake is increased over a barely adequate amount, much of the additional calcium is retained in the organism (164). Feeding of added calcium leads to a failure of resorption of the trabeculae of spongy bone (84), and consequently to an increase in the amount of such bone—an effect similar to that produced by the administration of estrogens. It appears that the organism has a means of conserving amounts of calcium not immediately needed, and that the storage of this element is under the influence of intake, parathyroid hormone, and estrogens, as well as of vitamin D.

Nutrition and Mineral Metabolism

Undernourishment.—The skeletal changes resulting from underfeeding in young guinea pigs, without specific dietary deficiencies, have been described (165). There is atrophy of the epiphyseal cartilage cells, and endochondral and periosteal bone formation proceed at a diminished rate, with return to an initial state of hyperactivity on refeeding.

Growth and development factors.—The occurrence of skeletal abnormalities in the offspring of rats reared on a diet deficient in many respects, and the prevention by a supplement of pig liver, have been reported (166).

Mineral metabolism.—In addition to the exhaustive reviews mentioned above in the introduction other reviews of mineral metbolism have appeared (167, 168). Only certain topics, of special interest in the physiology of bone, will be reviewed here.

Calcium deficiency.—The symptoms and pathology (169) and changes in chemical composition (170) in severe calcium deficiency in growing rats have been described. A form of calcium rickets, or osteoporosis is produced, with bones containing only about one half the normal amount of ash or calcium.

Magnesium.—The importance of the skeletal magnesium as a reserve for the organism is emphasized (171, 172). It is stated that magnesium leaves the bone salt crystal readily, but that its return to the crystal occurs only during redeposition of the bone salt.

Manganese.—The importance of manganese for the normal development of bone has been demonstrated for the chick (173, 174). In rachitic chicks (175) bone phosphatase was greatly increased; omission of manganese from the rachitogenic diet resulted in a decrease in bone phosphatase to about the normal level. The rat differs from the chick in being much less sensitive to a diet deficient in manganese (176), but pronounced manganese deficiency, produced in rats weaned without access to manganese, resulted in impaired growth (177).

Phosphorus.—The effect upon the tissues, including bone, of feeding diets extremely low in phosphorus to rats (178) and to dogs (51) has been studied. In rats, marked skeletal rarefaction occurred with accompanying rickets; in puppies there was extreme rickets, not demonstrably modified by simultaneous administration of vitamin D. The economy of phosphorus in the animal organism has been reviewed (15).

Cereals and rickets.—The history of twenty years work on the rachitogenic properties of cereal has been reviewed (179), with special attention to the most recent explanation of this action (180), i.e., that it is due not to the unavailability of the phosphorus of phytic acid (inositolhexaphosphoric acid), but to the fact that phytic acid prevents the absorption of calcium from the alimentary canal. The view that the phosphorus of phytic acid is poorly available to the rat, especially in the absence of vitamin D, is still held (181).

Fluorine.—In view of the evidence (16) that small amounts of fluorine exert a beneficial effect upon the teeth, there has been increasing interest in its presence in bones. In human bones there is, in general, a rise in fluorine content with increase in age (182), the lowest value found being 0.02 per cent and the highest 0.3 per cent, with no evidence of fluorosis. Rats fed small amounts of sodium fluoride take up fluorine in the bones; elimination from the bones follows upon withdrawal. When 300 parts per million of fluorine were incorporated into a rachitogenic diet the life span of rats was significantly increased (183). The pathological changes in bone, resulting from fluorine poisoning and characterized chiefly by sclerosis, have been described (184, 185).

Citrates.—The ability of citrates and other organic anions to protect animals against rickets (3) has been found not to apply to the chick (186).

Proteins.—Increase in the protein intake of rats (187) led to an increase in the total body calcium, proportional to the more rapid gain in weight induced by the extra protein; there was no consistent increase in the percentage of body calcium.

Vitamins

Vitamin A.—It is stated (9) that the prompt retardation of endochondral bone formation accompanying deficiency of vitamin A indicates a specific action, but no distinctive histological appearance has been found. Osteogenesis itself is not inhibited, as bone formation continues late in the deficiency. There are dramatic changes in bone as the result of hypervitaminosis A, and it is suggested (9) that an early consequence of excessive administration of vitamin A is acceleration of some processes of bone growth, including periosteal proliferation, epiphyseal cartilage sequences preliminary to endochondral bone formation, and remodelling of bone, attended by osteoclosis.

Vitamin C.—The changes in bone and teeth as a result of a deficient intake of ascorbic acid have been reviewed by active workers in the field (9). The lesions in growing animals are mainly the result of failure of formation of intercellular substances and the consequent weakening of supporting tissues. These changes result, in growing bone, in the failure of deposition of bone matrix and in transformation of osteoblasts to spindle-shaped cells. Earlier views of one of the authors of the review referred to, have been criticized (188) and are withdrawn (9).

Vitamin D.—Wolbach & Bessey (9), in reviewing the early tissue changes resulting from deficiency of vitamin D, state that "the first evidences of rickets are the failure of the cartilage cells to complete the cycle of cytomorphosis and the failure of the matrix lateral to the persistent cartilage cells to calcify." This reviewer (189) regards the first of these changes as dependent upon the second, and believes that all of the changes in the bones in rickets are secondary to a failure of calcification—not, it may be said, a new point of view. The pathology of rickets has been reviewed, with particular reference to the changes at the cartilage-shaft junctions (4).

The problem of the mode of action of vitamin D, in antirachitic doses, has been exhaustively considered (5, 6, 7, 19). It remains to be shown that, in these doses, this substance has any

direct or local effect upon the calcification mechanism of bone. On the other hand, large doses of certain activated sterols, including vitamin D, induce marked changes in bone (8), somewhat resembling those caused by parathryroid extract and associated with hypercalcemia. This action may be dissociated from the antirachitic action of vitamin D, since dihydrotachysterol, which is only slightly antirachitic, exhibits the calcemic effect and the effects upon bone (8). Relatively large doses of vitamin D, administered to young dogs previously fed a rachitogenic diet, produce a marked increase in the maximal rate of reabsorption of phosphate by the renal tubules, thus perhaps contributing to the rise in the level of inorganic phosphate in the plasma characteristic of the action of vitamin D (157).

Fractures

Callus formation.—The repair of bone includes callus formation and calcification of the callus, two processes which ordinarily occur simultaneously, but which may be separated under certain conditions (190, 191). The interrelations of these two processes will form the chief subject of this section.

After an initial reaction to the trauma of a fracture, the healing process begins. It consists essentially (52) of formation of a fibrocartilaginous callus, originating within the fracture gap, and of formation of new bone, originating at some distance from the fracture line and invading and replacing the fibrocartilaginous callus from without. Whether or not cells arising from the ordinary connective tissue of the callus have osteogenic potencies, cells arising from the deep or cambium layer of the periosteum and from endosteum, play the greater part in the formation of new bone. Bone seldom, if ever, arises spontaneously from within the callus. The process as a whole is an excellent example of organization of diverse means to a common end—the nature of the organization process itself is but little understood.

The early stages of this healing process are but little affected by the conditions which will lead to or prevent calcification (191). In the rat, the effects of failure of calcification begin to manifest themselves by about the fourth or fifth day following a fracture, at which time invasion of the fibrocartilaginous callus is under way under ordinary conditions. In the absence of calcification this invasion is delayed; the delay is comparable to the delay in

initiation of the rachitic type of cartilage removal in the epiphyseal cartilage following the onset of rickets. Healing of fractures in the rachitic rat may be further complicated by encapsulation of the callus cartilage in a dense mass of fibrous tissue or fibrocartilage. In such cases the x-ray may give the impression of nonunion (190). Eventually, in the rat, even in severe rickets, there is spontaneous calcification of the callus, leading to union of the fracture by partially calcified osteoid. All of these effects of noncalcification may be overcome by providing a diet favorable to the deposition of bone mineral.

These observations are contrary to the view (21) that "a local calcium excess" in the early callus stimulates osteogenesis. The general problem of the relation of the local presence of calcium salts to osteogenesis, in the healing of fractures, has been reviewed, and new experiments have been added (192).

Calcification of the callus.—In fractures in the rat, when maintained on an adequate diet, calcification in the intramembranous bone of the callus ordinarily occurs as rapidly as the matrix is laid down (52), as is the case in growing bone (49). In both cases, however, a lag may occur, attributed to local failure in mineral transport rather than to noncalcifiability of the matrix. In man (78) the lag appears to be the rule rather than the exception and is attributed to a rate of osteogenesis greater than the rate at which the calcification mechanism can operate, at the low level of serum phosphate in all individuals except infants and very young children.

The matrix of hyaline cartilage, in the callus in the rat, becomes calcifiable when the adjacent cartilage cells become vesicular or hypertrophic, but only matrix in contact with the invading bone calcifies (52). If the matrix has not been made calcifiable by hypertrophy of the adjacent cells, it calcifies only when converted into bone matrix by the advancing osteogenic process. Only tissues recognizable as bone matrix or cartilage matrix calcify in the callus; there is no random calcification in the fibrocartilaginous callus.

It has been contended that the chief source of mineral for the repair of fractures is from the dead bone at the site of the injury (193). Studies on the role of the local transfer of bone salt in rats, however, have led to the conclusion that the humoral source of calcium and phosphorus is essential for the normal progress of

healing, and cannot be entirely replaced by local sources of mineral without considerable delay in the healing of fractures (194). Similarly, studies of the healing of fractures in man (78) indicate that bone salt is transferred from the shaft to the callus during rarefaction of the callus-enclosed shaft, but that this process provides only a small contribution to the total calcium deposit within the callus.

It has been frequently stated that one or more of the serum calcium, serum phosphate, and serum phosphatase levels are elevated during the healing of fractures. A careful search of the literature, however, coupled with additional observations upon man (78) has led to the conclusion that there is no consistent significant elevation in any of these factors. As pointed out above, it is difficult to explain the calcification of a fracture callus, in an adult, with a phosphorus level in the plasma which is characteristic of rickets in the child.

It is known that the fracture callus is rich in phosphatase. Following the reasoning of Robison (79) the effect of administration of sodium betaglycerophosphate on the healing of fractures in rabbits has been investigated (195), with apparently favorable effects, even though the fractures in the control animals progressed to rapid healing. These experiments require confirmation with more objective and more adequately controlled observations.

Metabolic Disorders of Bone

Diet and osteodystrophy.—Changes in bone, produced by deficiencies of organic as well as inorganic constitutents of the diet, e.g., atrophy and osteoporosis produced in pigs by protein deficiency, and the relations of diet to osteodystrophy in domestic animals, have been reviewed (196).

Rickets.—Reference has been made to this subject above. New contributions have been made to its pathology (4). The acidosis of rickets, frequently cited as evidence for the view that rickets is a systemic disorder, has been attributed (197) to the fact that the phosphate available for excretion in the urine is not sufficient to carry out adequately the elimination of acid from the organism. The behavior of the organic acid-soluble forms of phosphorus in the organism in rickets has been reviewed (198).

Osteomalacia.—An extensive series of papers on osteomalacia in China has been reviewed (199), with the conclusion that the role

played by vitamin D in its pathogenesis is of primary importance; the importance of calcium, in both pathogenesis and therapy, is only secondary to that of vitamin D. Osteomalacia of the spinal column, resulting from deficient diet or from disease of the digestive tract, has been described (200 to 204). The known factors in osteomalacia have been reviewed (205).

Osteoporosis.—A postmenopausal form of osteoporosis and the osteoporosis of hyperthyroidism have been referred to above. Senile osteoporosis has been reviewed (206, 207) with special reference to the dietary factor and gastric function (208). Severe osteoporosis (perhaps osteomalacia) has been described (209) in association with long-continued steatorrhoea. An acute form of osteoporosis, simulating hyperparathyroidism, has been investigated (210).

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ENERGY METABOLISM

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The following review covers the available literature for the period from October, 1941, to September, 1942, inclusive, and is limited to considerations of the metabolism of intact warm-blooded animals.

Measures of nutritive energy of foods.—In the sense that the most conspicuous attribute of life is motion, that motion is an expression of energy, and that all nutrients essential to the life of animals are involved directly or indirectly in energy production, energy metabolism affords a significant point of view—a common denominator— in terms of which all nutrients may be studied and compared.

In regard to measures of nutritive energy the Special Committee on Evaluation of Feeds of the American Society of Animal Production reported, in part, as follows (1): Net energy (gross energy minus all losses and expenses of food utilization) is the ultimate measure of energy value, and thus commends itself especially for purposes of fundamental research under conditions of intensive experimental control.

The net energy values of rations and of individual foods, however, are not constants, since their metabolizable energy and heat increments are influenced by many factors including the plane of nutrition and the base value of heat production, the nutritive balance of the ration and the associative effects of ration components, the thermal environment, and the physical activity and endocrine functions of the experimental subjects.

The prominent effect of the plane of nutrition on the net availability of the metabolizable energy of a ration when the fasting heat production is used as the base value in determining the heat increment has been interpreted as resulting, in the main, from the sparing effect of the food in relation to the katabolism of body tissue nutrients. In this sense heat increments based on the heat production of fast do not express the real energy expense of food utilization.

Since the observed heat increments of rations for maintenance

are decidedly less than for body increase, the effect of this difference is to render net energy values for maintenance higher than for body increase. For convenience, however, net energy requirements for maintenance may be arbitrarily expressed in terms of net energy for growth or other production; but, whatever the plane of nutrition, the energy required for equilibrium will be increased as the environmental temperature is either above or below the limits of the zone of thermal neutrality—the total metabolizable energy of the food, therefore, becoming net in the sense of protecting the body from energy loss.

The utilization of the metabolizable energy of a ration for body increase, determined from a comparison of maintenance with supermaintenance planes of nutrition, is not much affected by the usual levels of food consumption. This is the preferred measure, therefore, for comparison of rations on the basis of net energy for growth and fattening.

The efficiency of utilization of the metabolizable energy of a ration has been found generally dependent on its nutritive balance; and the net energy of a ration is not necessarily equal to the sum of the separately determined net energy values of its component feeding stuffs. The associative effects of nutritive combination are unpredictable in the present state of knowledge. There is some evidence to the effect that in nutritively complete rations the utilization of the metabolizable energy for body increase and also for milk production approaches constancy. This subject deserves further investigation.

The above mentioned committee also analyzed the evaluation of foods on the basis of digestible and of metabolizable nutrients (2); and a notable series of mathematical studies (3 to 7) was made in Sweden, based largely on a critical review of Kellner's seventy-one energy metabolism experiments with steers, which leads to the proposal of a method of calculation of the metabolizable energy of foods for cattle from the contents of the foods in digestible nutrients and the use of factors as set forth in Table 1.

Forbes and associates have found (unpublished) that the metabolizable energy values obtained by the use of these factors generally compare well with results obtained by respiration colorimetry, but that Axelsson's factor for the metabolizable energy of the ether extract of roughages is decidedly too high to represent the low-carbon, ether-soluble acids of silages. The main question

in this relation is the significance of the digestible nutrient values from which metabolizable energy is computed—in consideration of the associative effects of feeding stuffs on their digestibility.

TABLE 1

FACTORS FOR CALCULATION OF METABOLIZABLE ENERGY OF FEEDING STUFFS FOR CATTLE FROM THEIR CONTENTS OF DIGESTIBLE NUTRIENTS*

Nutrients		Metabolizable energy per gm. digestible organic matter
		kcal.
Protein:	roughage	4.30
	concentrates	4.50
Ether extract:	roughage	7.80
	grains	8.30
	seeds of oil plants	8.80
	foods of animal origin	9.30
Carbohydrates:	polysaccharides	3.76
	trisaccharides	3.62
	disaccharides	3.56
	monosaccharides	3.38
	nitrogen-free extract (average)	3.70
	crude fiber	2.90

* From Axelsson (7).

This system, however, is already criticized (8), and the controversy bids fair to continue indefinitely, because the idea of energy values of individual foods, as constants, is, from a critical point of view, untenable.

Base values of heat production.—Any method of relating energy metabolism to the nutrients used to produce it depends, fundamentally, on the difference between the heat production at two planes of nutrition. The heat production at any plane of nutrition below the maximum can serve as the base value; thus the heat production of fast, of the so-called basal status, or of energy equilibrium or of multiples of this value, may serve as the basis of measurement of heat increments due to the food, but these increments differ with the plane of nutrition represented.

An effort to define the minimum base value of heat production

in animals was made (9) by the assumption of a division of the heat production of fast between net energy for internal work and energy expense of utilization of the body nutrients katabolized, the expense of this transformation being measured as the increase in heat production caused by feeding oleo oil and dried beef muscle (to represent the body nutrients katabolized during fast) to steers established in approximate energy equilibrium.

The heat increment and the net energy of this supplement were 26.5 and 73.5 per cent, respectively, of its metabolizable energy; and the net energy of the body nutrients katabolized during fast was designated the theoretical minimum base value of heat production.

This value, providing a base for expressing energy requirements for maintenance and for body increase in essentially the same terms, was tentatively found equivalent to 14.4 kcal. per kg. of live weight during fast and equal time standing and lying, or to 12.6 kcal. per kg. during fast in the lying position alone.

Dynamic effects of nutrients measured by procedures resulting in the sparing of body nutrients from katabolism, or implying the heat production of fast as a base value, are considered by the authors to be invalid. The heat production at or slightly above maintenance, therefore, is considered the most practicable base from which to measure dynamic effects in nutrition in general harmony with this investigation.

The efficiency of farm animals in the conversion of feeding stuffs to food for man.—The gross efficiency of the total livestock population of the United Kingdom to convert feeding stuffs into food for man was estimated at 4.1 per cent for dry matter, 8.7 per cent for energy, 11.1 per cent for protein, and 3.3 per cent for fat. This efficiency was found to vary with the species and the method of feeding (10).

Characteristics which determine the efficiency of swine as feed transformers are body temperature, skin temperature, pulse rate, basal metabolism, critical temperature, and dynamic effect of food. Measurements of the fasting metabolism of swine at temperatures from 11°C. to 24°C. show that these values are within the zone of thermal neutrality. The fasting metabolism of male pigs was measured as 20 per cent higher than that of females, but that of barrows was similar to that of females. The average fasting metabolism during summer months was found to be about 20 per

cent lower than during other months. The fasting metabolism of sows increases progressively during gestation. Among rations of the same general nutritive level the ration having the higher digestible protein content produced the higher heat stimulating effect (11). This generalization has not been found true for growing or for mature albino rats in seventy-day experiments, with unrestricted activity and equicaloric diets (12, 13, 14, 15). The loss of food energy in the form of methane is much less with swine than with ruminants. The efficiency of utilization of the metabolizable energy of a normal ration for swine was measured as 80.7 per cent for maintenance, 66.2 per cent for the growth and fattening of young pigs, and 71.5 per cent for the fattening of mature swine (16).

The utilization of cellulose by farm animals depends on its source, as determining the lignin coating of the fibers. In comparison with the energy value of 1 kg. of barley as a standard, the equivalent quantity of energy is produced by ruminants and horses from 1.2 kg. cellulose, by swine from 1.5 to 1.7 kg. cellulose, and by poultry from a still larger intake of this substance. Estimates of production requirement are for 1 kg. of milk, 1.2 kg. cellulose; for 1 kg. beef, 12 to 14.4 kg. cellulose; for 1 kg. mutton, 19.2 to 21.6 kg. cellulose; and for 1 kg. pork, 7.5 to 10.2 kg. of cellulose (17).

Basal metabolism.—First determinations of basal metabolism on inexperienced human subjects are usually 5 to 10 per cent higher than subsequent tests; and since most of the older standards of metabolism are based largely on first tests, they are 5 to 10 per cent too high. Determinations on normal, trained, healthy subjects average 5 to 8 per cent below the Aub-DuBois standards of 1917, or the Boothby, Berkson & Dunn standards of 1936, or 3 per cent lower than the Harris-Benedict standards. The Aub-DuBois standards are much too high for children, and the Bierring figures for the boys, and the Kestner-Knipping tables for girls, are more accurate for well trained subjects. On the average, the metabolism of women is 10 to 12 per cent lower than that of men of the same size, but in a cold environment it may be the same as that of men, and in a very warm environment may be 15 to 20 per cent lower (18).

The standard values for basal oxygen consumption in California adolescents appears to be lower than comparable values reported from other regions (19). The decrease in basal metabolism

during adolescence is greater in girls than in boys. From ages twelve to eighteen the metabolism of boys was observed to drop from 45.03 to 40.63 kcal. per sq. m. per hr., and of girls from 41.65 to 33.39 kcal. per sq. m. per hr.

In a study of the basal metabolism of normal infants from three to fifteen months of age the daily heat production was referred to age, weight, height, and surface area, but no one of these bases was found entirely satisfactory for the prediction of the metabolic rate (20). The basal metabolic rate of twins was no more nearly the same than that of the nontwin infants.

A new direct respiration calorimeter for measuring the heat loss of premature infants, which is said to be simple, inexpensive, accurate, and easy to operate, has been devised (21).

With regard to metabolism in old age, experimental observations on Adolf Magnus-Levy at the age of seventy-six compared with twenty-six revealed decreases in oxygen utilized per minute of 24 per cent, in weight of 11 per cent, in surface area of 6 per cent, in calories heat produced per hour of 22 per cent, in calories per kg. per hour of 12 per cent, and in calories per sq. m. per hour of 17 per cent (22).

Insensible perspiration is greatly diminished in old persons (aged ninety to one hundred and ten); it varies from 9 to 25 gm. hourly; basal metabolism is decreased to 20 to 30 cal. per sq. inch hourly (23).

Of probable significance in relation to basal metabolism are determinations which were made of the vital capacity of 744 men. There was a decrease in vital capacity from 3150 cc. at forty to forty-four years to 2400 cc. at ninety to ninety-four years. The causes were loss of elasticity of the pulmonary tissue, lessened mobility of the thoracic cage, and diminished muscular power (24).

Two reports have dealt with the effect of climate on basal metabolism. In 78 white immigrants in Batavia the basal metabolism averaged 5.98 per cent less than normal. In 18 white students native to the Dutch Indies the basal metabolism averaged -1.7 per cent, while in two mountain villages in Java determinations of basal metabolism averaged $+8.73$ per cent (25). The basal metabolic rate on normal male Chinese and natives at Sourabaia averaged 9 per cent below the standard of Boothby, Berkson & Dunn. Since normal male Europeans in Batavia showed a similar reduc-

tion of basal metabolic rate, this reduction was not racial, but was due, in the main, to the tropical environment (26).

The basal metabolism of rabbits was found to be affected by the environmental temperature to which they were adapted. When such determinations are to be used as measures of the effect of superimposed conditions, the previous environmental temperature should be maintained at 28° to 29°, and the rabbit should be habituated to this temperature for three weeks prior to the measurements (27).

With regard to the basal metabolic rate in disease, in one thousand cases pulse rate, tachycardia, thyroid enlargement, exophthalmos, "nervousness," tremor, palpitation, and underweight increased in varying degrees with increases in basal metabolism, while fatigue and overweight produced effects of opposite trend. The same trends were not found in association with increases in the level of total metabolism (28).

The mean metabolic rate of 166 persons with low-grade chronic illness, probably secondary to chronic infection, was −8. Of these persons 83 per cent had rates of 0 or below, and 43 per cent had rates of −10 or lower. Persons with moderately severe and severe symptoms had significantly lower rates than did those with mild symptoms (29).

Heat production was measured in divers pathological conditions characterized by muscular wasting. Previous to puberty no significant relation of heat production to muscular wasting was observed, but in adults muscular wasting was associated with relatively slight or moderate decrease in heat production except in myotonia atrophica in which the decrease in heat production was sometimes greater than were the accompanying changes in muscular mass and function. The findings in this condition are postulated as being related to changes in organs other than the muscles (30).

A possible interrelationship between arteriosclerosis and hypothyroidism is suggested by the observation that subjects exhibiting arteriosclerosis manifest greater incidence of hypometabolism than do those without arteriosclerotic manifestations (31).

With overweight human subjects on a diet of 1200 kcal., loss of weight was accompanied by a drop of 16 per cent in oxygen consumption, an increase of 24 per cent in arteriovenous difference, and a drop of 30 per cent in the cardiac output (32).

The results from a study of over 1000 cases indicate that, in the absence of infection, subnormal body temperature is a better index for thyroid therapy than is the metabolic rate (33).

The basal metabolic rate determined approximately one-half hour after smoking may be either above or below previous control determinations, possibly as affected by individual differences in the responsiveness of the sympathetic system and of the adrenals (34).

The possibility of using the insensible loss in weight as a basis of computation of the basal or other heat production has received further study. With trained subjects, under strictly defined conditions, an empirical relationship was derived for making such computations. Under the prescribed conditions the relationship of insensible loss to heat is fairly constant if the average of a series of determinations, rather than a single one, is employed (35).

The relation between the basal metabolism and the endogenous nitrogen excretion, which normally approaches constancy, seems not to be fundamentally a constant since it is found susceptible of alteration by increase in the basal metabolism as a result of the height of the previous caloric intake, and by increase in the excretion of endogenous nitrogen as a result of previous exposure to low environmental temperature (36).

In a test of total respiratory metabolism of the albino rat during thirty-six hours following the withdrawal of food the post-ingestion maximum heat production of 954 kcal. per sq. m. per 24 hrs. dropped to 78 per cent of this value at the twentieth fasting hour. During the next eight hours the curve was elevated in semblance of a dynamic effect of fat, with maximum of 84 per cent at the twenty-second hour and final return to 76.6 per cent by the twenty-eighth hour. Thereafter, until thirty-six hours, the general trend is horizontal with oscillation about the value of 78 per cent previously reached at the twentieth hour. An R.Q. of 0.70 was first reached at the twenty-eighth hour (37).

A study of the resting energy metabolism and quantity of air exhaled in relation to body weight of growing Jersey cattle, compared with the basal metabolism in growing man, led to observations in part as follows:

From birth to about six months the resting energy metabolism of Jersey heifers varied with the 0.84 power of body weight (kcal. per day = 88.3 Wt. in kg.$^{0.84}$); from six to twenty-five months the resting metabolism varied with the 0.56 power of body weight

(kcal. per day = 295 Wt. in kg.$^{0.56}$), with exactly the same power found to relate surface area to body weight (sq. m. surface area = 0.15 Wt. in kg.$^{0.56}$). The resting metabolism per sq. m. increased from about 1300 kcal. at birth to 2000 kcal. at six months at which level it remained up to twenty-five months, the middle of the first gestation period. This contrasts with the basal metabolism of mature animals of various species which tends to vary with the 0.73 power of body weight (kcal. day = 70.5 Wt. in kg.$^{0.73}$). The resting heat production in terms of total digestible nutrients was approximately one-half the total digestible nutrients consumed by these growing cattle. The metabolism-weight curve in man is similar to that of cattle; in both there is a tendency toward direct proportionality of metabolism to weight prior to weaning, and for a 0.6 power proportionality following weaning (38).

A study of the resting maintenance cost of growing dairy cattle indicates that prior to about five months the metabolism tends to vary directly with the body weight (about 24 kcal. per pound per day); following this age the metabolism varies not with $W^{1.0}$, but with $W^{0.6}$—a fact which signifies that increasing the body weight 1 per cent increases the maintenance cost only 0.6 per cent (39).

In rats, rabbits, and sheep the metabolic rates of liver slices per unit weight of tissue were found to decrease in the same way that the metabolic rates per unit weight of the intact animals decrease as the body weight of the species increases (40). Liver slices from a cow and horse did not conform to the prediction equation derived for the smaller species.

In further consideration (41) of this principle the weight-specific rate of heat production in the intact animal and the respiratory rate of the excised tissues of the same animal were calculated to bear the same relation to the body size of the animal, i.e. $Q_{O_2} = KW^{-.23}$. From this it was deduced that the relative intensity of respiration (of intact animal or of tissues) is proportional to the relative body weight of the species, and that the relative rates of respiration of excised tissues preserve this characteristic relation. Thus, oxygen consumption plotted against body size on a logarithmic scale (placing both on a relative basis) would yield a series of parallel straight lines, one for each tissue and one for the intact animal.

Nutrient metabolism.—Carbohydrate combustion in human subjects was found to be the same after intravenous as after oral ad-

ministration of dextrose (42). In normal persons or those suffering from spontaneous hypoglycemia the rate of carbohydrate combustion appears to be independent of the level of blood sugar. Hyperglycemia induced by epinephrine does not increase the R.Q., whereas hyperglycemia induced by dextrose does increase the R.Q. and the carbohydrate combustion (43, 44).

A disturbed carbohydrate metabolism in rats maintained more than a year on a fat-deficient diet has been observed (45). After six months on the fat-deficient diet the rats became deficient in a fat-contained factor that is necessary for the normal metabolism of carbohydrate. At this time the R.Q.'s during carbohydrate assimilation were abnormally high. After a year or more the R.Q.'s were no longer high, and this result was attributed to the counter-effect of a lack of a normal amount or of normal functioning of insulin.

Respiratory quotients of protein metabolism in a Dalmatian dog (46) were found to be considerably below 0.8. The R.Q.'s were estimated by assuming a factor for the oxygen consumed per gram of urinary nitrogen in the metabolism of protein, and a normal fat metabolism. However, since the use of the usual respiratory factors (for carbon dioxide and oxygen) lead to abnormally low nonprotein R.Q.'s which the authors considered incorrect, the assumption that only one of these factors is in error, and the other correct, seems questionable; and since in most cases the protein metabolism was a small fraction of the total metabolism, all possible errors in assuming a certain constant R.Q. for the larger non-protein portion are related to the former smaller fraction—which might lead to abnormal results. No definite explanation of the low respiratory quotients is offered, and they can not be accounted for as resulting from the elimination of nitrogen as uric acid. The method of calculation did not reveal the true respiration factors for the Dalmatian dog.

It has been shown that the rapidity of development and the degree of ketosis reached during fast are inversely related to the protein content (47) and the nature of the protein of the preceding diet (48), which determine the amount of "stored" protein available for katabolism and formation of antiketogenic residues. Various natural fats fed prior to fasting influenced the degree of ketosis only insofar as they affected protein katabolism.

The energy of the body gain, as protein and fat, was found to be

decreased and the total heat production was increased in rats on a diet in which the chloride ion was deficient (49).

Respiration trials with chicks indicated that flavin deficiency decreased the utilization of protein but did not affect the utilization of food energy beyond the effect of greatly decreased appetite (50).

Dynamic effects of nutrients.—The dynamic effects of carbohydrate and fat, or of carbohydrate and protein, were found to be less than the sums of the separately determined dynamic effects of these combined nutrients, whereas the dynamic effect of protein and fat is nearly equal to the sum of the individual calorigenic effects (51). Dynamic effects of food were found much less in adipose subjects than in normal controls (52). In experiments with premature infants phenylalanine and tyrosine did not increase the heat production, whereas glycine caused significant increases. It is suggested that the energy producing reactions contributing to the dynamic effect of tyrosine and phenylalanine in adults supplant a different series of reactions in infants (53).

Exercise.—Three papers, during the past year, on glycine in relation to muscular endurance, bring to a total of fourteen the recent contributions on this subject that have come to the writers' attention, and serve to continue the conflicting testimony that has characterized the series as a whole. The problem would be a difficult one to solve with certainty, and no one of the fourteen papers reports a background of complete balances of matter and energy on nutritively complete equicaloric diets differing only in glycine or in gelatine.

A bicycle ergometer experiment seemed to show that the ingestion of glycine increased the energy output of women by 22 per cent and of men by 32 per cent. Glycine given with urea is said to have produced an increase of 79 per cent in the energy output of men, but an irregular increase varying from 16 to 62 per cent in women. The glycine was given, or the glycine and urea were given, in wine. After a similar amount of wine alone the energy output was decreased (54).

A second report of similar experiments, including 921 control and 1834 experimental tests with 75 medical students as subjects, led to the general conclusion that each individual has a natural energy output which can be increased by the ingestion of amino acids, vitamins, and phosphates, though during the whole study

20 per cent of the students showed no increased watt-minute output (55).

In a study of strength of manual grip as affected by glycine eight subjects were tested twice daily for eleven weeks. The improvement in grip strength in subjects receiving glycine was no greater than that of those who were given placebos. Neither creatine nor creatinine showed changes attributable to the ingestion of glycine (56).

In a study of the vitamin supplementation of the U. S. Army rations it was concluded that no useful purpose, as measured by standardized severe exercise on a motor-driven treadmill, would be served by the enrichment of present U. S. Army garrison rations in thiamin chloride, riboflavin, nicotinic acid, pyridoxin, pantothenic acid, and ascorbic acid. In neither brief, extreme exercise nor in prolonged, severe exercise and semi-starvation were there indications of any effects, favorable or otherwise, of the vitamin supplementation on muscular ability, endurance, resistance to fatigue, or recovery from exertion. Healthy young men expending an average of 3700 to 4200 kcal. per day are not benefited by a daily supply of more than 1.7 mg. thiamin chloride, 2.4 mg. riboflavin, and 70 mg. ascorbic acid (57).

Similar results were obtained by a different technique(58). Thiamin, cocarboxylase, riboflavin, and parenteral vitamin B complex given intravenously had no immediate influence on recovery from voluntary muscular fatigue on a bicycle ergometer by subjects receiving "adequate" nutrition. Recovery from fatigue was measured in double work periods—the value of working ability ten minutes after working to fatigue is expressed as percentage of the working ability in the initial period to give the per cent recovery. The use of double work periods in the study of fatigue and the influence of caffeine on recovery had been described previously. Caffeine-sodium benzoate (0.5 gm.) had no significant objective effect on recovery (59).

The physiological factors concerned in the physical capacity for sustained heavy exercise were studied in subjects working to exhaustion against a periodically increasing load on a bicycle ergometer. Heart rate, total ventilation, oxygen consumption, and R.Q. were found to increase linearly with the work-load. The slope of the regression line and the ultimate level reached were individual variables. As exhaustion approached the metabolic and circulatory

functions showed no definite responses, but total ventilation was often markedly increased, while the alveolar carbon dioxide tension and the carbon dioxide percentage in the expired air declined sharply. The latter factors were the most reliable objective signals for the onset of exhaustion. The individual capacity for exhaustive exercise was dependent on the ability of the subject to maintain a high oxygen consumption and on the efficiency with which the work was performed. The relative importance of these two factors varied with the individual subject although, in general, both increased directly as the work-performance increased (60).

In a study of training, and its effects on man at rest and at work, a group of fourteen subjects trained for middle-distance running during six months. The training regime was accompanied by a slight increase in weight, a decrease in resting pulse rate of five beats per minute, a slight decline in respiratory rate and volume, and a slight increase in plasma chloride. An increase in efficiency of grade walking was observed. In exhausting work there was an increased capacity for supplying oxygen to the tissues and greater utilization of anaerobic energy reserves. The work increment unaccounted for by these alterations presumably results from a more economical organization of body functions. The increased capacity for accumulating lactic acid that is developed during training, and the notably high lactate levels attained by first-class athletes, point to lactate level as a useful index of cardiovascular fitness (61).

In a comparison between the physiologic responses of men and women to moderate and to strenuous exercise on a motor driven treadmill the trained were differentiated from the untrained by more economical ventilation during exertion, ability to attain a greater maximum ventilation, greater mechanical efficiency as measured in terms of lower oxygen consumption for a given amount of external work, ability to attain a greater maximum oxygen consumption, lower gross R.Q. during exercise, lower blood lactate for a given amount of exercise, ability to push self to a higher lactate before exhaustion, less increase in pulse rate for submaximal exertion, and quicker recovery in pulse rate following activity (62).

Adaptation of white men and negroes to prolonged work in humid heat was investigated in experiments in which the subjects walked for two hours on a motor driven treadmill, the metabolism being elevated to seven times its basal level. The tests were con-

ducted in the summer in Mississippi, where room temperatures averaged 31°C. and humidity 79 per cent. The test on partially acclimatized northern white men raised the body temperature to intolerable levels, increased their heart rates to nearly maximal values, and forced most of the men to discontinue the walk before the end of the two hours. Negro sharecroppers who were acclimatized by field work performed the walk without marked elevation of body temperature or heart rate, and they sweated less but drank more water than did the northern whites. White sharecroppers in Mississippi, also accustomed to field work, were intermediate between the other groups in adaptability to the conditions of the experiment. The superiority of the negro sharecroppers was related to lower energy requirements in performing the walk. Negro servants in Mississippi were no more successful than northern whites in regulating body temperature, but were able to sweat at much higher rates (63).

In a similar test of negro and white sharecroppers in comparison with northern whites, by means of a standard walk and running to exhaustion on a motor driven treadmill, both races exhibited results as follows, which were related to advancing age from eight to twenty-four years: Downward trends of heart rate and of oxygen requirement in moderate work, and of lung ventilation and respiration in both moderate and exhausting work, and upward trends of blood lactic acid and of R.Q. in the exhausting run. The negro sharecroppers performed moderate work with slightly greater efficiency and with lower heart rates than did the whites, but their respiratory rates were slightly higher. In exhausting runs the whites breathed more slowly, and thus used a larger fraction of the vital capacity as tidal air. The maximal oxygen consumption during exhausting work was slightly greater in the northern whites. There was a negative correlation between ability to consume oxygen and maximal heart rate (64).

The influence of variation in work and metabolism on the manner of heat loss from the unclothed human subject at constant or varying environmental temperatures have been investigated (65). With a constant environmental temperature the skin temperature of the working subject remained practically constant as the load was increased, and consequently the heat loss by radiation and convection was unrelated to the extra metabolism. Evaporative cooling with increasing sweat secretion balanced the extra heat resulting

from increasing work. Variations in environmental temperature had no relation to the heat production resulting from work, but the avenues of heat loss were shifted somewhat.

The effect of body size on energy exchange in work was studied in two men weighing 99 kgm. and 44 kgm., respectively, who ran or walked on a motor-driven treadmill at rates which raised the metabolism to seven to eleven times the basal level. The efficiencies of the two men in performing the work were the same, but the larger man accumulated heat under conditions under which the smaller man attained heat balance—heat production being in proportion to body weight, but the ratios of weight to surface area, and heat production to surface area, were about 20 per cent greater for the larger man (66).

A study of the energy expended by horses in drawing loads with varying resistance and at different speeds was conducted at the New Hampshire Agricultural Experiment Station (67).

The action of exercise on ketosis has been investigated in rats and man. With rats in a state of ketosis a short bout of heavy exercise causes an immediate drop in the blood ketone level. During a period of three to four hours thereafter there is a phase of overproduction of ketone bodies so that the blood values for exercised animals go to higher levels than in controls. In man on a ketogenic diet ketone compounds of blood and urine are diminished during heavy but not during light exercise. During the subsequent rest period their excretion was increased. These results support the view that in states of ketosis exercise increases the oxidation of ketone bodies, and also causes the liver to produce them at an increased rate (68, 69).

Energy metabolism of diving animals.—The conservation of the oxygen reserve of the seal while diving depends on quickly coordinated mechanisms involving rapid onset of bradycardia, selective restriction of peripheral circulation, and reduction of the metabolic rate in some tissues (70). Muscle metabolism appears to be aerobic during the first part of the dive, but anaerobic metabolism (lactic acid formation) predominates in the latter part. Muscles appear to be isolated from the circulation during diving since muscle hemoglobin is completely reduced when the blood is still half saturated with oxygen, and the lactic acid which accumulates in the muscles during the dive does not enter the blood stream until the recovery processes have started (71). Total muscle me-

tabolism is not decreased during the dive, and may actually be increased. In connection with diving there does not seem to be any lower, basal limit for metabolism since it can be readily depressed during the dive and promptly restored during recovery (72).

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THE RESPIRATORY SYSTEM[1]

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A reviewer in war time suffers from several handicaps. He receives confidential articles which he can not quote and he does not receive journals in which there are articles which he would like to quote. Therefore, this reviewer will have no difficulty in keeping well within the limits set by the Editors of the Annual Review of Physiology for this year. The literature quoted is, with one exception, from American sources, published mainly in the American Journal of Physiology. It divides itself into two groups, the neural control of respiration and the chemical control with its concomitant problem, anoxia.

Neural Control of Respiration

Central localization.—Pitts, Magoun & Ranson (1) outlined two discrete divisions of the recticular formation extending caudally from the level of the facial nucleus over the cephalic four fifths of the inferior olivary nucleus. From one of these divisions these authors elicited coordinated respiratory acts while from the other subdivision expiratory responses were obtained on stimulation. This work indicated that there was functional subdivision of the respiratory center into an inspiratory and expiratory center.

These conclusions did not pass unchallenged. Brookhart (2) claimed that the results were due to the high voltage used in stimulation. Gesell (3) suggested that the results were due to stimulation of afferent fibers exerting a predominant influence on inspiration or expiration, respectively. Both of these objections have been answered satisfactorily by Magoun and Pitts. Beaton & Magoun (4) have shown in the monkey both with high voltage (8.7 to 13.7 volts) and with low voltage (0.9 to 5.6 volts) stimula-

[1] The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department.

tions that there are two discrete regions: the one controlling inspiratory activity, the other expiratory. In a second paper, Magoun & Beaton (5) report repetition of their work on the cat. Again, using voltages ranging from 0.9 to 7.4 volts for stimulations, they found that the excitable reticular function could be subdivided into a dorsal expiratory and a ventral inspiratory zone. In order to answer Gesell's objection that their results were due to stimulation of afferent fibers, these authors made intracranial sections of the glossopharyngeal and vagal roots on one side. Comparison of the results of stimulation of this side with the unoperated side revealed the same results as described above. These results, combined with those previously reported, enabled the authors to state that the responses to medullary stimulation were not due to activation of afferent pathways to the reticular function either from the pons, from the spinal cord, or from the vagal or glossopharyngeal nerves. Pitts (6), also, carefully repeated and extended his former work and was able to show that the functional subdivision of the respiratory center into an inspiratory and expiratory center was a correct physiological subdivision.

Therefore, it can be concluded that the respiratory center is divided into two portions, the one controlling inspiration and the other controlling expiration.

Reflex control.—Gesell & Hamilton (7) have made a study of the respiratory responses resulting from stimulation of several afferent nerves. Stimulation of the superior laryngeal nerve gave an expiratory response, stimulation of the vagus gave an expiratory drive while stimulation of Hering's nerve was generally followed by an inspiratory act. Stimulation of the saphenous nerve produced a rapid, rhythmic form of breathing in which both inspiration and expiration were increased. From these results, Gesell & Hamilton divided afferent respiratory nerves into those which carry predominantly impulses affecting (*a*) inspiration, (*b*) expiration, and (*c*) mixed.

A closer analysis of this problem has been made by Hodes & Larrabee (8). The superior laryngeal nerve was stimulated while records were made of impulses in a single efferent fiber of the phrenic nerve. Stimulation of the superior laryngeal nerve during inspiration suppressed the impulses in the phrenic nerve. The duration of the pause increased with the number of stimuli until

inspiratory activity stopped completely. It is obvious from this work that the superior laryngeal nerve could be classed more exactly as an inhibitor to inspiration rather than as an expiratory nerve as in Gesell's classification. Experiments similar to those of Hodes & Larrabee should be conducted with Hering's nerve, the vagus, and the saphenous nerves.

Gesell, Brassfield & Hamilton (9) have observed an after-discharge of the respiratory center following stimulation of Hering's nerve and the saphenous nerve in the dog. The duration of this after-discharge was inversely proportional to the length of stimulation. These authors postulate that the alkalinity produced by the hyperventilation increases the destruction of acetylcholine in the cells of the respiratory center and thus decreases the after-discharge following the longer periods of stimulation. It may be sometime before definite evidence of this postulate will be forthcoming.

Gesell & Moyer (10) have investigated the reflex respiratory response resulting from open pneumothorax. The first effect of pneumothorax was to produce marked inspiratory activity associated with weak expiratory activity. Later, there was an increase in intensity of both inspiratory and expiratory activity in which expiratory activity predominated. These authors concluded that the inspiratory act at the beginning of the pneumothorax is due to a vagal inspiratory drive which has replaced an expiratory reflex. The secondary acceleration of breathing is explained by an increased asphyxial chemical drive acting on the expiratory centers. An electrical analysis of this problem should be made in order to get a more exact explanation of this complex problem.

Comroe & Schmidt (11) have investigated the respiratory acts resulting from movements of the limbs which have been isolated vascularly. Electrical stimulation of the lumbar anterior spinal roots as well as passive movements of legs produced an increase in rate of respiration. This respiratory effect was abolished by denervation. Passive movement of the leg in normal man increased respiratory activity. Strong chemical stimulation produced similar respiratory changes but the stimuli used were outside physiological limits. It is obvious that these proprioceptive reflexes play an important role in the increased respiratory responses during exercise.

Chemical Control of Respiration

Dumke, Schmidt & Chiodi (12) have continued their work on the chemoreceptors by studying the effect of 100 per cent oxygen, oxygen with 3.5 per cent carbon dioxide, and 10 to 14 per cent oxygen with 3.5 per cent carbon dioxide in nitrogen on dogs before and after denervation of the aortic and carotid chemoreceptors. The newer results of this work were that the addition of 3.5 per cent carbon dioxide in 10 per cent oxygen before chemoreceptor denervation increased the pulmonary ventilation more than 3.5 per cent carbon dioxide alone. Following denervation, pulmonary ventilation is decreased below the normal response to 3.5 per cent carbon dioxide in 10 per cent oxygen. Therefore, the effect of anoxia on the response of the center to carbon dioxide is a depressant action. Shock & Soley (13) have observed a similar finding in man for they have shown that the reduction of the oxygen content in the inspired air from 17 to 12 per cent decreases the respiratory response to carbon dioxide in certain subjects. Therefore, at lowered barometric pressure, carbon dioxide may not be an efficient respiratory stimulant.

Moyer & Beecher (14) have reported the interesting finding that stimulation of respiration will occur if anoxia is prolonged sufficiently in an animal under anesthesia with the known peripheral chemoreceptive and vagal proprioceptive reflex mechanisms denervated. This stimulation followed a primary depression of respiration and was characterized by periodicity and rapid rate. Watt, Dumke & Comroe (15) report that in trained dogs, the inhalation of 100 per cent oxygen caused depression of respiration while after chemoreceptor denervation, oxygen inhalation either increased respiration or had no effect. These two types of experiments demonstrate that the oxygen content of the blood may modify respiratory response directly through the respiratory center although the effects are secondary and of no importance in comparison with the primary responses through the peripheral chemoreceptors.

Anoxia

Gellhorn, Cortell & Carlson (16) have continued their work on the action of anoxia on the various centers of the brain. Previous results of many investigators have shown that anoxia depresses

cortical processes while it excites respiratory and vascular responses. This difference is due to the reflex activity of the chemoreceptors on the respiratory and vasomotor centers. When the peripheral receptors are removed, the direct action of anoxia on the respiratory and vasomotor centers is a depressant effect. In this paper, the authors have shown that the hypothalamic, medullary, and sympathetic centers have increased excitability during anoxia with regard to autonomic activity while somatic activity is decreased. However, on denervation of the carotid sinus regions and after double vagotomy, the pressor response of the blood pressure is converted into a depressor response on medullary or hypothalamic stimulation. This result again demonstrates that the response of the vasomotor centers to direct stimulation is conditioned by afferent impulses coming from the peripheral chemoreceptors.

Van Harreveld (17) carried out an interesting series of experiments on the survival time of central synaptic conduction following asphyxiation. In this work, a section of the spinal cord was asphyxiated for twenty-five to thirty-five minutes by injecting Ringer's solution into a ligated portion of the dural cavity under pressure higher than the blood pressure. From two days to four weeks after such an operation, action potentials were recorded in the anterior roots under asphyxiation. This asphyxia was produced by either cutting the abdominal aorta or by the administration of nitrogen. The author describes the surprising result that a previous period of asphyxiation increased the survival time of the reflex action potentials. The increased survival time was not present the first few days after the primary asphyxiation but developed during the second to the fourth week. Van Harreveld concludes that this result is due to an increased resistance of synaptic conduction to asphyxiation, even though the initial asphyxiation destroyed a large number of ganglion cells. It would be very important to determine if less drastic initial anoxia, such as exposure to 10 per cent oxygen, would produce the same result. Fazekas, Alexander & Himwich (18) have determined the survival time of newborn and adults of various species to complete anoxia. They describe that an adult rat dies after one and one-half minutes in pure nitrogen while a rat on one day of age survives fifty minutes. The time of survival decreases with age, for at seventeen days the time of survival is similar to that of an

adult. There is a marked difference in this phenomenon, for the period of tolerance is very short for the guinea pig and long for the rat. These results are not surprising when consideration is given to the partial degree of anoxia in which the fetus develops. The tissues retain their ability to maintain an anaerobic existence some time after birth. Himwich (19) and his associates have continued this work by studying the sources of energy for this anaerobic survival of the newborn. They showed that by injecting insulin into the newborn, causing a hypoglycemia, the survival time was decreased. Also, the injection of iodoacetate and fluoride cut down the survival time of the newborn. The giving of glucose to eight-day old rats increased their average survival time from sixteen to thirty minutes. These results emphasize the importance of carbohydrate stores in the survival of these young animals during anoxia.

The importance of the interaction of carbohydrate metabolism and brain anoxia has again been demonstrated by Gellhorn & Kessler (20). The effects of anoxia on brain potentials are increased during insulin hypoglycemia. The action of the hypoglycemia can be alleviated by the inhalation of pure oxygen. These results demonstrate that the metabolic activity of the brain is dependent on two components: the supply of glucose and the supply of oxygen. If one is diminished and the other increased, the metabolic needs may be met; while, if both are diminished, the additive effect is one of increased depression.

Northrup & Van Liere (21) have continued their work on the absorption of metabolites from the intestine under various degrees of anoxia. They placed isotonic solutions of glucose and glycine in loops of intestines and measured the absorption over a period of ninety minutes. At a simulated altitude of 28,000 feet, there was no change in the absorption of glucose. At this altitude, however, a decrease in the absorption of glycine occurred. The authors conclude that the absorption of glycine may involve an oxidative process which is impaired at high altitudes.

Lawson (22) reported that sulfanilamide increased the tolerance of rabbits to altitude. He claimed that this improvement in altitude tolerance was associated with a retention of carbon dioxide in the blood. It is obvious from these results and other unpublished work that the practice of grounding an aviator from ten days to two weeks following treatment with sulfanilamide is not based

upon experimental findings. The length of time that a man should be grounded following the sulfanilamide or sulfathiazole should be given careful experimental study. Dill & Hall (23) have published their results on arterial oxygen and carbon dioxide at high altitudes. They found that at simulated altitudes as high as 44,000 feet, the arterial saturation varied from 68.9 per cent to 74.9 per cent while breathing 100 per cent oxygen. The carbon dioxide pressure fell slightly with an equivalent rise in the pH. They conclude that, during breathing of oxygen at 40,000 feet, there is no increase in ventilation but, above that level, anoxia produces deeper breathing. This hyperventilation reduces the carbon dioxide pressure in the lungs which enables a man to withstand altitudes of 44,000 feet. The latter altitude corresponds to 18,000 feet while breathing air of ordinary composition. Diffusion of oxygen by physical laws is sufficient to explain the saturation of the blood with oxygen both during work and rest at these extreme altitudes.

Gemmill (24) has published the results obtained through a study of 2521 cadets subjected to a simulated altitude of 18,000 feet without extra oxygen in a low pressure chamber at Pensacola, Florida. At this altitude, 208 needed oxygen before the end of the fifteen minute stay at that altitude; 34 lost consciousness. Brombacher (25) has reprinted his standard altitude-pressure tables which are invaluable in low pressure chamber work.

Carbon Monoxide Anoxia

Maurer (26, 27) has studied the effects of carbon monoxide anoxemia on cervical lymph flow and cerebrospinal fluid pressure. Dogs and cats exposed to 0.5 per cent carbon monoxide developed an increased production of cervical lymph. This effect was reversible, for inhalation of 100 per cent oxygen returned the lymph flow to normal. Similar concentration of carbon monoxide increased the cerebrospinal fluid pressure 1.74 times the normal. This change was attributed to the increased cerebral blood pressure and the increased permeability of the cerebral capillaries. Anoxia, produced by decreasing the concentration of oxygen in the inspired air, gave the same result as carbon monoxide.

Chiodi, Dill, Consolazio & Horvath (28) studied the respiratory responses of dogs and men subjected to acute carbon monoxide

poisoning. No increased ventilation was seen in either dogs or men during carbon monoxide anoxia. The only effect was a depression of respiration. Since the chemoreceptors depend on their stimulation from a fall in pressure of oxygen, they are not stimulated by carbon monoxide anoxia, in which the partial pressure of oxygen does not fall.

Mechanical Changes

Dean & Visscher (29) have made a thorough study of the pressure-volume changes during respiration in dogs. From these results, they are able to evaluate the viscous and elastic components of respiration. The viscous resistance produced by an obstruction which produces turbulence is reduced when helium oxygen mixtures are substituted for air. This results from the decrease in turbulence in the helium-oxygen flow in comparison to the turbulence caused by nitrogen-oxygen flow.

Books

Two books in this field have appeared in the past year: Van Liere's (30) text on "Anoxia" and Hoff & Fulton's (31) "Bibliography of Aviation Medicine." The former is a valuable reference text on anoxia although the author does not clearly distinguish between the effects of acute and chronic anoxia in some of his chapters. The latter book has several sections on anoxia, respiration, and related subjects which give valuable references in these fields.

Summary

It is obvious from this short review that two main lines of attack are being used at the present time in the field of respiration: the neurological and the anoxic. A tremendous interest is being taken in the latter in view of its practical nature in aviation. However, it must be kept in mind that only when an aviator goes above 35,000 feet while breathing pure oxygen does he become anoxic. Even at 40,000 feet, his anoxic symptoms are very slight. Therefore, anoxia is a very small problem in aviation as long as the aviator is supplied with adequate oyxgen. The results of many investigators in the field of anoxia and in the methods of supplying oxygen to the aviator cannot be published until the war is over. It may

be said, however, that many advances in physiology of respiration will be made during these crucial war years. A review of these advances a few years from now will reveal this rapid progress.

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MUSCLE

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On account of the limited space, this review deals mainly with those problems of muscle physiology upon which some interest is centered at the moment and excludes all papers of purely biochemical content. An excellent collection of various discussion and review articles covering nearly all problems of muscle physiology has been published at the beginning of the review period (1 to 13).

The Structural Pattern of Muscle and the Physicochemical Process of Contraction

Dubuisson (14) and Fischer (8) attempt to integrate our incomplete knowledge concerning various physicochemical changes during contraction, such as changes in pH, in volume, in birefringence, in x-ray spectrogram, in impedance, in light permeability and scattering, in thermoelasticity, and in viscoelasticity, into a unitary concept of all these contraction phenomena. Both concepts, which have much in common, are still rather hypothetical in parts but offer starting points for further research.

Cross-striation and contraction.—Carey (15) summarizes his earlier findings concerning the inconsistency of the cross-striation of muscle fibers and offers further evidence (16) on fixed frog muscle that with increase in temperature the cross striae are multiplied. On living muscles of a fresh water shrimp, he believes to have observed complete reversibility of the increase in number of cross-striations with temperature (17). "The sarcomere as a constant unit is a morphologic myth" to him, and he believes the cross-striation to be the expression of the functional state of the fibers, comparable to bands of inorganic precipitates in gelatine-filled micro-capillaries, a model in which the spacing of the bands depends on chemical concentrations, heat, and light influences. Speidel & Jordan (18) are convinced from their own experimental experience that in various striated muscles, including those of shrimps, the number of sarcomeres remains constant. They point out that examination of Carey's photomicrographs does not reveal

inconsistency of the sarcomeres, and that Carey was misled by telophragmatic doubling or tripling of the dark stria of the A-disc. For isolated single frog muscle fibers, earlier reports that under isometric condition the A-disc shortens and the I-disc lengthens have been confirmed with improved techniques (19, 20). At rest, A has a much lower modulus of elasticity than I, but in the active state the elasticity moduli are lowered, especially for the I substance. The changes in the relative widths of the discs have been observed also for crustacean muscles (21).

Birefringence and contraction.—There exists a positive correlation between the degree of birefringence and the contractile power per weight unit of comparable normal muscles (22). Furthermore, diminution in birefringence for various types of atrophy parallels the diminution in contractile power per weight unit, thus indicating how essential the submicroscopical pattern is for proper function of the contractile mechanism.

The total birefringence of muscle is partly due to the parallel arrangement of submicroscopical micellae and partly due to the crystalline structure of the latter. Buchthal & Knappeis (20), studying the diffraction pattern of single muscle fibers, confirm a previous suggestion that the micellae bundles in the resting fiber are not arranged completely parallel, but that either stretch or isometric contraction produces full parallel orientation. The flow birefringence of myosin solutions, which is due to the shape and structure of the micellae or myosin particles, can be diminished significantly and reversibly by adenylic pyrophosphate (23), one of the most important substances involved in the working metabolism of the muscle. This influence of the adenylic compound on the myosin birefringence is of special interest since the enzyme adenylpyrophosphatase is either myosin itself or some protein very closely associated with it (24, 25, 26), and since no other substances have been found which diminish myosin flow birefringence reversibly (27). Myosin "particles" prepared from rabbit, octopus, or ray muscle have all about the same length and same proportion length to width (28).

X-ray spectrography.—The x-ray spectrogram and the elastic properties of myosin resemble closely those of keratin that have suffered a breakdown of the cross-linkages of the polypeptide grids (29, 30). The supercontraction of myosin is not due to a sim-

ple reorientation of the long thin units but involves a further folding and close-packing of the polypeptide chains. Astbury & Bell (31) propose a scheme which explains such a close-packing of the sidechains and which satisfies the physical data.

Volume changes.—Ernst & Mórocz (32) found the volume decrease during a short tetanic contraction just equal to the theoretical volume constriction due to ionization of that amount of potassium which is released from an indiffusible state during the tetanus. In a veratrine poisoned muscle, both the volume decrease and the potassium release are prolonged to the same extent.

The Energy Exchange in Muscle

Resting muscle.—Stannard (33) concludes that the resting respiratory mechanism is quite different from that during activity since both mechanisms can be inhibited by different chemicals. The resting metabolism does not involve the cytochrome-cytochrome oxidase system, and the dehydrogenases are not the same in both mechanisms. The activity enzymatic mechanism probably includes a copper-containing enzyme which is not a part of the resting mechanism. As in various other cells, the resting respiration is less sensitive to narcotics than the activity respiration (34).

It is unnecessary to assume that the diminished oxygen consumption of a mammalian limb after sympathetic stimulation is due to a direct action on the resting metabolism since such a diminution occurs always when the blood flow or the oxygen saturation is reduced below critical values (35). In frogs, however, after curarization and in the absence of circulation, sympathetic stimulation increases considerably the resting heat liberation (36).

Active muscle.—Brown, in a remarkable discussion of the thermodynamics of muscle activity (7) based on both older and newer observations (37), identifies five different processes: (*a*) an initial, almost isothermic reaction associated with the activation of the myosin linkages; (*b*) a process yielding the shortening heat, the rate and magnitude of which depends on the rate and extent of shortening; (*c*) a process yielding the maintenance heat and responsible for stabilization of the linkages; (*d*) an isothermic process accompanying and equal to the work done, stoichiometrically related to the oxidative recovery process; (*e*) a process accompanying lengthening. Brown assumes as the underlying mechanism a

crystalline protein structure, in which the active linkages are maintained in the resting state by a chemical system some component of which is built into the linkages. The latter is activated by stimulation and, due to release of a catalyst, energy liberation ensues until stabilization of the linkages occurs with simultaneous refixation of the catalyst to the linkages. All these assumptions are reasonable in the light of our present knowledge about the probable identity of adenylphosphatase with myosin and its activation by calcium ions (26).

The various concepts of the chemistry of contraction are discussed by Meyerhof (9), who upholds the classical view, and by Sacks (38), who assumes that normally the work of the muscle is based initially on fundamental oxidative processes, and that aerobic recovery heat and oxygen consumption are due to the "chemical inertia" of the stimulated muscle which does not return instantaneously to its resting metabolism. Under anaerobic condition, the primary reaction is lactic acid formation, the latter being neutralized by phosphocreatine hydrolysis; whenever the lactic acid mechanism is inadequate, formation of hexosemonophosphate initiates supplementary sources of energy (39). That in the muscle of the diving seal lactic acid formation only starts after five to ten minutes, at a time when the muscle hemoglobin is completely reduced (40), can be interpreted in favor of Sacks' view.

D. K. Hill measured with improved technique the oxidative and the anaerobic delayed heat of frog muscle at 0°C. (41) and concludes from his analysis that the early rapid phase of the anaerobic delayed heat must be attributed to the breakdown and not to the resynthesis of phosphocreatine. Measuring the pH of frog muscles with normal and with chemically inhibited respiratory systems with the carbon dioxide exchange method, the same author (42) is convinced that there is no possibility that lactic acid is identical with the substrate normally oxidized in aerobic activity. The lactic acid formation represents only a branch reaction, which is independent of the main channel of oxidation.

Careful measurements with the glass electrode of the changes occurring during stretch and release of the resting or active muscle indicate that only the later pH changes during activity are due solely to chemical changes while the earlier changes are partly caused by a shift of the isoelectric point of the myosin (43).

Although it is still generally accepted (44) that the ultimate

fuel for muscular exercise is carbohydrates, the fact that muscles from frogs kept for a long time at low temperatures do not break down glycogen and often do not form lactic acid under anaerobic condition (45) may indicate the existence of other sources of energy. All attempts to increase muscular work by special diet or vitamins seem to have failed. A few investigators still claim that gelatin or glycine increases the strength in man, but a large number of well-controlled experiments did not detect any beneficial effect (e.g. 46, 47). Vitamin C might increase activity of the isolated muscle (48), but its influence upon physical efficiency in man is doubtful (49). Of all drugs, only caffeine has been reported to cause an objective effect upon the work output in man (50).

Permeability and Electrolyte Distribution

Loss of potassium during activity.—In contrast to the generally accepted view that muscular activity leads to a considerable loss of muscle potassium in exchange for sodium (51), Miller & Darrow (52) found that in rats swimming for one hour no loss of potassium per unit nonfat solids occurs despite an increase in serum potassium. They accounted for the latter increase by loss of glycogen from the muscle with simultaneous release of sufficient potassium to maintain a constant ratio of potassium to nonfat solids inside the muscle. In perfused frog legs, epinephrine decreases considerably the potassium loss during activity but has no influence upon potassium accumulation by the muscle after potassium chloride injections (53). Like frog muscle, toad muscle releases more potassium after direct stimulation than after indirect stimulation; the potassium loss for direct stimulation is diminished in muscles denervated fifteen to twenty days previously; acetylcholine produces in denervated muscles about thirteen times the normal potassium release (54).

Potassium and fatigue.—Potassium chloride injection delays muscular fatigue and restores the working capacity of a fatigued muscle (55). Fatigued rat muscles with a low potassium and a high sodium content have a higher oxygen consumption *in vitro* than normal muscle (56); this is claimed to be due to the change in mineral content since corresponding changes in oxygen uptake can be produced by injection of potassium or sodium into normal rats.

Potassium accumulation in muscle.—Normally the mammalian muscle fibers contain only 12.7 m. eq. of sodium but 145.0 m. eq. of potassium (57). Boyle & Conway (58) investigated intensively the potassium uptake and loss by frog sartorii immersed in various salt solutions suitably adjusted to avoid volume changes. Potassium, as chloride, can be accumulated; this fact indicates that the membrane is permeable to cations and anions. However, sodium, due to its greater ion diameter in solution, cannot permeate the muscle membrane. The authors propose equations for a membrane system based on consideration of the osmotic, electric, and Donnan equilibria, which satisfactorily predict the experimental results concerning muscle volume, internal potassium concentration, and electrical potential when the external concentrations, total internal indiffusable molecular concentration, and the differences between the total negative and positive charges of these molecules are known. The potassium in muscle is held by the electrostatic charges of the indiffusable anions. The fact that muscles can take up potassium against a concentration gradient anaerobically as well as aerobically (59) supports an explanation of potassium uptake due only to a membrane equilibrium.

Despite large variations of the muscle potassium in rats, the sera of which have fairly constant potassium and sodium levels, injection of potassium chloride will produce in all muscles only a transient increase in potassium unaccompanied by any change in sodium concentration (60). Rats, swimming after injection of labelled potassium, accumulate in their muscles four times as much K^{42} as resting rats (61), and most of the muscle potassium is apparently present in a nondiffusible state as also indicated by other experiments (62). Noonan *et al.* (63), using labelled potassium, found for rats a fivefold increase in potassium uptake on stimulation and a twofold increase one hour after denervation, but no increase in stimulated frog muscles. They attribute the increase in penetration observed in rats solely to increased circulation, which was absent in their frog experiments. Lyman (64), however, found increased potassium penetration also in denervated mammalian muscle in which increased circulation was avoided by cutting the ventral and dorsal roots within the vertebral canal. The penetration of K^{4} is only moderate for normal muscle in comparison to other tissues, but after equilibrium is established, the bulk

of the radioactive potassium is stored where normally the bulk of the total body potassium is found, i.e., in the muscles (63). Any excess of potassium in the serum is probably quickly taken up by the viscera and then slowly released to be stored in the muscles (65).

Relation between potassium and other ions.—The interchange of ammonium and potassium is a pure membrane effect and not due to energy expenditure (66, 67). As long as the experimental calcium concentration does not change the permeability of the membrane, a certain amount of potassium of the muscle may be exchanged for calcium (68). Magnesium and potassium can compete for places in the muscle fiber, and magnesium behaves like potassium under the influence of anoxia, pH, and ammonium chloride (69).

Intracellular and extracellular spaces.—The general scheme of dividing muscle into two spaces has its limitation but is useful. From a histological point of view, the intracellular spaces are the muscle fibers enclosed in their sarcolemma, and the extracellular spaces contain the vascular system, the connective tissue, the lymphatic system, and the extracellular fluid. In dogs, this extracellular space accounts for about 15.5 per cent of the muscle volume (70). When it was assumed the muscle fiber does not contain chloride, it was believed that the chloride space gives an accurate measure of the extracellular space. Dean (71) finds, however, about 15 m. eq. of chloride in isolated muscle fibers but emphasizes evidences indicating that most, if not all, of this chloride is on the surface of the fibers. Boyle *et al.* (72) calculate from their experiments that sodium chloride outside the fibers corresponds to 12 to 13 per cent of the muscle volume while the total chloride space is about 14 per cent. Heilbrunn & Hamilton (73), however, believe that with the usual methods of chloride determination some of the chloride inside the fibers is lost, and with a new method they claim values of 41.5 m. eq. for the isolated fiber and 51.5 m. eq. for the whole muscle. According to these values, the extracellular space would be 25 per cent, about twice the space shown histologically. Hemoglobin solutions penetrate only into the vascular system (74), but sugar and inulin penetrate farther although the sugar space (75) and the inulin space (72) are smaller than the chloride space. According to Conway & Fitzgerald (76), the major part of the difference between

inulin and chloride permeation arises from the presence of some chloride within the fibers and is related to the potassium concentration by an equality of the products of these ion concentrations on each side of the membrane (58). Using radioactive sodium, chloride, and phosphate (77 to 79), it was confirmed that only a small part, if any, of the sodium or the chloride may be normally intracellular, and that Na^{24} and Cl^{38} does not penetrate into the fiber while phosphate permeation is unlimited but slow. According to Wallace *et al.* (80), the muscle membrane is impermeable for bicarbonate ions.

Theories of electrolyte equilibrium.—Donnan (81) supports Boyle and Conway's theory (58) of a pure membrane equilibrium and emphasizes that by the assumption of the formation inside the fiber of an impermeable complex anion, which includes potassium, an accumulation of potassium can occur against a diffusion and electrical gradient without the need for any outside energy supply. Some new evidence (82) has been offered in support of Mont and Netter's theory that normally the muscle is permeable to potassium and hydrogen and impermeable to chloride and sodium, and that only during activity sodium can be exchanged directly with potassium while at rest sodium can exchange only with hydrogen. Steinbach (83) points out the complexity of the mechanism involved in electrolye equilibrium across the muscle membrane, which according to many indications is quite permeable to all ions. He assumes that a main factor in maintenance of the equilibrium is the energy release and the "make-up" of the whole protoplasm. Further evidence (84) for this theory, which is often referred to as the pump theory, has been found in smooth muscles of invertebrates, which are said to accumulate potassium to a large extent without much cation exchange or simultaneous entering of anions. Dean (12), who discusses carefully all these three theories, favors the pump mechanism since he believes the latter can best account for all the experimental facts.

Injury potential.—In frog muscle, the magnitude of the injury potential is controlled by the concentration of potassium chloride at the injured as well as at the uninjured surface. The experimental potentials can be explained by diffusion potentials if one assumes that the membrane normally has a low electrolyte concentration, which increases after injury (85).

Mechanical Properties

Single fiber.—Ramsey & Street (1) found also for single fibers that the form of the twitch is dependent on which end the fiber was stimulated and that "Funk'sche Nase" may occur in such twitches. Enhancement after tetanus was found, too, in single fiber preparations. If a single fiber is allowed to shorten more than 70 per cent, its relaxation becomes incomplete and its elasticity constants are changed. In single fibers, fatigue sets in rather suddenly and excitability disappears completely (86). An isolated but intact fiber [illegible] extension with at least twice the force than that which co[illegible] counted for by the elasticity of the sarcolemma (87).

Swelling and temperature influence.—Frog muscles immersed in Ringer's solution swell less when stretched; also, swelling is less at increased temperatures (88). Swelling decreases and shrinking increases the strength of the isometric twitch. Sudden temperature increases have a twofold influence upon muscle strength: a quick acting factor tends to decrease the force while a slowly acting factor tends to increase it. The latter factor is absent in muscle under stretch.

Smooth muscle.—The lengthening of isolated uterine muscle and of the muscle of perfused blood vessels under a load follows the same time course as relaxation after contraction, thus indicating that both processes are controlled by the same, probably viscous, forces (89). For smooth muscles of various invertebrates, these two curves are both exponential, but release of tension after stretch occurs more slowly (90).

Action Potentials

A good review of the action potentials of muscles has been written by Young (6), who upholds the classical theory of the origin of the potential but admits the influence of the motor end-plate potentials upon some of the action-current records. Spike potential amplitude and tension may vary independently, and none of the various slow potential changes following the spike is invariably correlated in time with contraction (91). The number of peaks found in the monophasic action potential records of twitches of muscles composed of a mixture of slow and fast fibers seems to

have no definite relation to the number of peaks in the isometric myogram; there exists no strict separation in slow and fast fibers (92).

Goldberg & Eyster (93) measured the potential distribution on the surface of frog muscles half immersed in Ringer's solution. Mathematical analysis and comparison of their numerous records for each single twitch brought about by nerve stimulation are interpreted as indicating that the potential changes are of a "polar nature"; regions of positive and negative potentials develop coincidentally, undergoing growth and decline.

Myoneural Junction

Distribution and histological changes.—Katz & Kuffler (94) demonstrated that nearly all sartorius fibers are innervated by two, some even by three motor endplates. Approximately one out of eleven endplate regions are supplied by two nerves (95). Carey (96) claims pleomorphism for the endplates because he found in fixed material that relaxed fibers are associated with retracted endplates while in more contracted fibers the endplates are expanded, consisting of thinner nerve fibers. Acute inanition furthers this "ameboid motion" of the endplate (97).

Endplate potential (*e.p.p.*).—The existence of the e.p.p. has become well established by the work of three independent groups of investigators under the leadership of Schaefer (98), Feng (5), and Eccles (13). However, in many details there is still discrepancy, even in papers from the same group. There is no doubt that the e.p.p. must be regarded as a local depolarization, reaching in mammalian muscle its maximum after 2.5 msec. and disappearing completely after 5 to 6 msec. (95, 99). The e.p.p. mechanism is only slightly fatigable (98, 100), and the e.p.p. corresponds to 10 to 40 per cent of the spike potential (99, 101). During Wedensky inhibition the e.p.p. is not altered although the mechanical changes are completely lacking (98, 100) or present only as local contractions (5). During the refractory phase of the muscle fiber, the e.p.p. is diminished and only local muscle responses are elicited (102, 103). Curare depresses the e.p.p. but does not abolish it (100, 104, 105, 106). In curare poisoning, the e.p.p. spreads with 50 to 70 per cent decrement per mm. (107). The safety factor for stimulation of the muscle fiber by the e.p.p. is high since the critical level,

below which the e.p.p. must be diminished to be unable to elicit a spike potential, is rather low (104, 107).

In partial curare poisoning the e.p.p. produced by a single nerve volley may cause (*a*) a full propagated impulse, (*b*) an abortive propagation extinguished after about one millimeter, or (*c*) an abortive propagation, which then suddenly flares up to the fully propagated type (103). A second volley produces an e.p.p. of three times the potential of the first e.p.p. (100), and this explains well the early stage of facilitation in partly curarized muscle (108). Physostigmine prolongs the e.p.p. in normal (100, 103) and in curarized muscles (105) and may also produce local contractions (5).

Drug action.—Mammalian extrinsic eye muscles are exceptionally sensitive to physostogmine (103, 109); and facilitation during partial curarization can be demonstrated easily. In physostigmine poisoning, a single nerve volley evokes a regular series of spike potentials, but repetitive nerve stimulation or arterial injection of acetylcholine causes a local contracture under the endplates, which blocks the propagation of excitation (108). In frogs, physostigmine may affect either the response to a first nerve volley or only that to a second volley; it also prolongs the minimum interval at which two nerve volleys elicit a summated muscle response (110).

Feng and co-workers observed that in amphibian (111) and in mammalian muscle (112) after physostigmine, guanidine, barium, or veratrine, the discharges at the motor endplates produce antidromic impulses through the efferent nerve fibers. These physostigmine, but not the veratrine, action-currents of the ventral roots are missing after curare. The interpretation of the recorded action-currents as antidromic conduction of the endplate discharges can be questioned since Lloyd (113) observed that after stimulation of normal muscles by a motor root volley, centripetal volleys ensue not only in the ventral but also in the dorsal roots. These impulses are initiated just at the time of the ascending phase of the muscle potential and may be due to a stimulation of intramuscular nerve fibers by the muscle action-current.

Action-current methods have been used for quantitative determination of drug action upon neuromuscular transmission in human subjects (114). Curare produces complete paralysis only in doses causing bulbar signs (115). Quinine methylchloride and erythroidine produce partial curarization (114, 115). Acetylcholine

intra-arterially causes a profound motor stimulation, followed by severe pain and transient paralysis, while prostigmine produces prolonged paralysis for voluntary action, innumerable fasciculations, and repetitive responses to single electrical nerve stimulation (116).

Excitability

The problem of excitability with special consideration of the various mathematical treatments proposed has been discussed by Blair (2), who assumes as the simplest and most likely model of the excitable system a single polarizable membrane with equal electrical capacity per unit area at the regions of current entry and exit, but with much higher electrical resistance per unit area at the cathode. The general form of the strength-duration curve contains two exponential terms due to this differential property of anode and cathode.

A new method, using three electrodes, has been introduced to measure *in situ* the true α-excitability of the muscle fibers, themselves (117 to 119). Lapicque (120) reports evidence indicating that "isochronism or quasi-isochronism" between nerve and its muscle exists as long as the nerve chronaxie is not diminished by subordination under the central nervous system. For the same reason, only in relaxed human subjects can stable chronaxie values be found for the various muscles (121).

Isolated fibers from a whole frog muscle, which has been immersed in glucose solution, are still irritable to electrical current, but only local contraction occurs since conduction has been lost (122). Osmotic pressure changes between certain limits sensitize the toad sartorius to cold and to electrical stimulation (123). Intensive ultraviolet flashes of microseconds' duration, which kill infusoria instantaneously, do not affect the excitation mechanism of muscle but produce contracture after a latent period of a few seconds (124).

Muscular Activity and Adrenals

In adrenalectomized rats on a diet rich in sodium, no weight loss occurs, but the work output of the muscle is still below normal levels (125). Isolated muscles from adrenalectomized frogs (126) or rats (127) fatigue much quicker but produce at the beginning the same tension as muscles from sham-operated animals. Removal of

the adrenal medulla alone does not influence the work output in rats (128). Glucose combined with sodium chloride injections improves the work of adrenalectomized rats to the level of adrenal demedullated animals, and this improvement is not prevented by simultaneous administration of potassium chloride (129). Adrenalectomized cats, kept alive by desoxycorticosterone, have normal muscle force and excitability (130).

In the muscle of adrenalectomized rats, the phosphagen and the adenylic pyrophosphate content is normal, but hexosediphosphate is distinctly diminished (131), apparently because the muscles are less able to esterify phosphoric acid. Desoxycorticosterone restores normal phosphorylation, even *in vitro* (132). Muscles of adrenalectomized animals, although they contain more potassium than normal muscles, lose less potassium during work or after acetylcholine injection (133). This has been explained by the absence of hexosediphosphate formation needed for the glycogen metabolism with which the potassium release is probably linked. In adrenalectomized rats, for every 10 m.eq. of potassium stored in the muscle, 3 m.eq. of magnesium are lost, and sodium chloride enters the muscle easily, which explains why not only desoxycorticosterone but also injection of sodium chloride prevents the potassium storage (134). In normal dogs, desoxycorticosterone produces attacks of muscle weakness, which is associated with a partial replacement of the intramuscular potassium by sodium. Both the muscle weakness and the cation exchange may be prevented by potassium chloride administration (135).

Atrophy, Dystrophy, and Other Muscular Disorders

Atrophy.—In atrophy due to lower motor neuron lesions, the weight loss of rat muscles continues still at a considerable rate after two weeks, but in upper neuron lesions the equally severe weight loss stops at this time and the spastic muscles start to regain weight (136, 22). Fibrillation is absent in upper neuron lesions; the increase in acetylcholine sensitivity is small and transient (136); the muscle force per weight unit never diminishes appreciably; and also, birefringence does not decrease as in denervation atrophy (22). In atrophies brought on by denervation, cast, or tenotomy, the glycogen content of the muscles diminishes before

appreciable weight loss occurs (137), and in all three types of atrophy the oxygen consumption of the muscle is increased (138). Creatine decreases in denervated muscles (139) and increases when reinnervation occurs (140). Of all the known chemical processes of muscular activity, only glycogen synthesis and creatine phosphorylation are diminished in denervation atrophy (139, 141). There is rapid and complete restoration of the capacity for these processes after reinnervation. Before complete regeneration is accomplished, the glycogen is often temporarily maintained above normal levels (137). Denervation is followed by a pronounced increase in the capacity of the muscle to store labelled phospholipids. This change occurs before appreciable weight loss and continues for weeks (142, 143).

The fibrillations in denervated muscles are conducted contractions through the whole length of the fibers and may originate according to Eccles (144) from foci often other than the endplates, while Hayes *et al.* (145) found them always spreading from the endplate regions. If muscles are isolated from all afferent impulses and higher centers but their motor neurons left intact, disuse atrophy occurs with a weight loss about equal to that in denervation atrophy, but no fibrillation can be observed, and excitability and muscle force are less impaired (146, 147, 148).

In denervation atrophy, quinine does not retard the weight loss although it prevents fibrillation by decreasing the hypersensitivity to acetylcholine and potassium (149, 150). Of various drugs tested, Levine *et al.* (151) found only prostigmine and atropine of significant influence upon the course of denervation atrophy. However, the atrophy-accelerating and fibrillation-increasing influence of prostigmine has not been observed by others (152) in experiments with doses only somewhat smaller, and the atrophy-retarding and fibrillation-decreasing effect of atropine may be only a simulated one due to the cachectic inanition of the atropine-treated rats (153).

In well-controlled animal experiments, it has been observed that daily, weak, galvanic stimulation retards only slightly the weight loss of denervation atrophy (154) while strong and prolonged galvanic or faradic stimulation applied daily retards considerably the weight loss and also accelerates the return of the muscle to its initial volume after reinnervation (155). In disuse atrophy, faradic stimulation decreases the weight loss considerably

but rather independently of the duration of the daily stimulation, which varied from two seconds to two hours (147).

Dystrophy.—The fact that a vitamin E-free diet produces muscular dystrophy in certain animals has been confirmed and the minimum requirement established, which is the same for natural vitamin E and for synthetic alpha-tocopherol (156). Oral administration is more effective in curing this experimental dystrophy than parenteral administration (157). The dystrophies occasionally observed in vitamin A-deficient rats are probably due to a simultaneous lack of vitamin E (158). Muscle, dystrophic due to E deficiency, has a higher oxygen consumption (159) and higher chloride content (160) than normal muscle. Tocopherol restores quickly, even *in vitro*, normal metabolic level (160, 161), but the muscle chloride remains somewhat elevated. In rabbits, cod liver oil counteracts the antidystrophic action of tocopherol (162). In rats with only mild dystropic symptoms, thiamin in relatively large doses is able to cure the dystrophy (163).

Although Cabrera (164) observed in an experiment upon himself an apparent dystrophic condition due to avitaminosis E which was promptly cured by tocopherol, it is more and more recognized that in patients with muscular dystrophy or related disturbances, vitamin E is not able to produce a cure. While some authors (165, 166) still report some beneficial effects, the majority, most of them using objective methods in determining muscular improvement, agree that tocopherol and other vitamins may occasionally improve the subjective symptoms but do not improve the dystrophic condition of the muscles (167 to 171).

Myasthenia gravis.—Further reports (11, 172, 173) indicate that myasthenia gravis patients benefit from extirpation or x-ray treatment of the thymus, but the mechanism by which this disease is linked with the thymus function is completely unknown, and no experimental material concerning this question has yet been published. The characteristic changes in the electromyogram of myasthenia are identical with those of normal subjects after partial curarization (174, 175, 176), and are completely, but only temporarily, relieved by prostigmine. Only in some cases with very advanced symptoms is prostigmine unable to restore normal muscle strength. In contrast to normal subjects, acetylcholine injection does not produce muscle weakness but causes sustained contraction in myasthenia (176) while epinephrine causes only moderate

return of muscle power as in partially curarized normal subjects (177).

Myotonia.—In myotonia of goats (11) and of man (178, 179), a single impulse arriving through the nerve leads to repeated conducted activity of the motor units. Potassium sensitivity of the muscles is increased, at least in goat's myotonia, by depressing the elevated potassium sensitivity (11). In myotonia of man, not only such muscular factors cause the myotonic symptoms, but also a reflex mechanism is involved due to afferent impulses starting from the contracting muscle (178). This reflex spasm is not restricted to the prime mover but may occur also in other muscles of the same limb (179).

Smooth Muscle

Vertebrate smooth muscle.—It is more and more realized that the term "smooth muscle" covers a group of different contractile structures, rather heterogenous in their physiologic behavior (4). Therefore, no general statements concerning excitability, conduction, validity of the all-or-none rule, and drug action should be made. In different smooth muscles, the coordination of the individual muscle fibers might be effected solely through nervous control, or by syncytial connection between the muscle fibers, or by both mechanisms to a greater or lesser extent (3).

The fact that stimulation of the minute nerves supplying the muscles of small blood vessels produces spatially limited vascular reaction suggests for this type of smooth muscle an arrangement in motor units comparable to those in skeletal muscle (180). In the nictitating membrane of the cat, when some of the postganglionic fibers are severed, only a localized part of the muscle shows hypersensitivity to epinephrine and changes in its electrical excitability; these findings indicate that in this muscle, too, a certain division in units exists (181). The action currents of this muscle as well as those of the pilomotors have been studied by Rosenblueth & del Pozo (182), who examined the relation between electrical and mechanical events.

Both in intestinal and uterine smooth muscle, a persistent weak contraction is often due to asynchronous activity of different parts of the muscle, and the strength of contraction is dependent on the frequency of discharges and their duration (3). These discharges, which are conducted and follow the all-or-none rule, show a great

diversity in different muscles. The monophasic potential is either a brief spike or a long continued negativity (183). Sympathetic stimulation or epinephrine has a twofold effect: diminution of excitability, which can lead to block, and a tendency to set up muscular impulses. Bozler (184) found, furthermore, at the site of the pacemakers in these muscles a characteristic weak activity, recognizable as a local potential much smaller than the spike potentials. These local potentials always precede and apparently initiate the spontaneous contractions. If the muscle excitability is decreased by epinephrine, only the local potential arises and the conducted ones are missing.

It is well known that the motility of the uterus *in situ* is dependent on the different phases of the sexual cycle. The isolated cornu uteri of a rat in estrus has a lower resistance to extension that that from a rat in anestrus, but the cervical part of the uterus has its strongest tonus in estrus (185). The burst of spike potentials accompanying contractions are more pronounced in the cat uterus during estrus than during anestrus (186). The responses of the isolated cat uterus to electrical stimulation are local during anestrus and conducted during estrus. Injection of estrone in cats during anestrus increases the electrical excitability of the isolated uterus, and the responses become conducted more and more with increased duration of treatment (187).

Invertebrate smooth muscle.—In smooth muscle of invertebrates, there may be found, besides normal excitatory nerve fibers, four other types of nerves: (*a*) inhibitory fibers, (*b*) fibers conditioning the reaction to excitatory and inhibitory impulses, (*c*) regulatory fibers, which replace excitatory and inhibitory innervations if an inherent automatism of the muscle exists, and (*d*) shortening and lengthening fibers for muscles which have no true resting length and in which lengthening and shortening are the only active phases (10). These complicated innervation mechanisms are best known at the moment for crustacean muscles. The "opener" of the claw receives one set of excitatory fibers but two different sets of inhibitory fibers. The "common inhibitor" reduces muscle activity without influencing the muscle action currents while the "true inhibitor" also reduces considerably the action currents (188). The "closer" of the claw has only one, the common inhibitor, but two sets of motor fibers, which cause either a fast or a slow contraction with different types of action currents (189). Heat measurements

revealed that for the slow contraction the efficiency of the muscle is about twice that of the fast one (190). By direct stimulation of such muscles, one may excite either type of contraction or both simultaneously; thus repetitive stimulation often produces rhythmic twitches superimposed on a slow contraction (191). Neither atropine nor curare affects crustacean muscle while epinephrine and some other drugs increase its excitability and contractility (192).

The slow adductor muscle of the scallops receives some true inhibitor nerve fibers, which are responsible for the quick reflex relaxations (193).

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THE DIGESTIVE SYSTEM[1]

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Salivary secretion.—By electrical exploration of the medulla in anesthetized cats Chatfield (1) found that stimulation of various points, situated mostly in the reticular formation and in regions of intramedullary course of the facial or glossopharyngeal nerves, resulted in salivation. Points which gave predominantly submaxillary salivation lay mostly rostral and medial to the middle third of the facial nucleus. Points, primarily concerned with parotid salivation, were concentrated caudal to and above the submaxillary centre and medial to and above the cranial end of the nucleus ambiguus. Both ipsilateral and bilateral salivation were obtained from unilateral stimulation, with or without accompanying elevation of blood pressure. The optimal frequency for electrical stimulation of the submaxillary gland of cats through the chorda tympani was found by Wills (2) to be about nine per second. Pilocarpine stimulated the submaxillary gland to a greater salivary flow for a given blood flow than did stimulation of the chorda. The potassium concentration of saliva was found to increase markedly at rates of secretion below 0.03 ml. per min. During stimulation by pilocarpine the submaxillary glands of dogs and cats gained water and sodium while losing potassium and the saliva had a higher concentration of sodium, chlorine, lactic acid, and greater carbon dioxide tension than the saliva produced by excitation of the chord tympani (3). Certain aspects of the calcium and phosphorus content of human saliva have been studied by Becks & Wainwright (4). Hellauer & Schneider (5) found by stimulating the chorda tympani in dogs that the protein content of saliva increased with elevation in velocity of salivary flow when such increased salivation was effected by a rise in the voltage of the stimulating current but not by increasing the frequency of stimuli. The salt content in these experiments changed directly as the velocity of salivation. These workers considered the chorda as containing some fibres which caused secretion of mucus and others, salt. Barnum & Armstrong (6) gave to a patient by stomach tube a solution of sodium

[1] This review includes the available literature from July 1, 1941, to July 1, 1942.

phosphate containing radioactive phosphorus. The maximum concentration of radioactive phosphorus in saliva was reached between three and twenty-four hours but never equalled that in the plasma.

Gastric secretion.—Using dogs with a transplanted fundic pouch and a pouch of the remainder of the stomach, Gregory & Ivy (7) presented evidence that one humoral stimulus was liberated from the gastric mucosa by the presence of secretogogues and another from the mucosa of the small intestine by the presence therein of food or secretogogues. In one dog the pyloric region of the gastric mucosa was not essential for the demonstration of the humoral response of the transplant to the gastric component. Gray, Wells & Ivy (8) found a continuous hypersecretion of highly acid gastric juice in dogs after either removal of the small intestine or exclusion of gastric, pancreatic, and biliary secretions from the small intestine. This hypersecretion appeared to be related mainly to the stimulating effects of surgical intervention rather than to a release from enterogastrone. Loew & Chickering (9) injected subcutaneously into dogs with Heidenhain pouches the histamine antagonist, thymoxyethyldiethylamine. This agent did not induce gastric secretion and, after histamine was also injected, gastric secretion was markedly increased in volume and acidity over that obtained with histamine alone. During the rapid secretion of hydrochloric acid by the gastric mucosa of a dog's stomach pouch, Mann, Grindlay & Mann (10) observed only occasionally an appreciable difference in the chloride concentration of the arterial and gastric venous blood. The difference was mainly in the cell chloride. Gray & Adkinson (11) mounted the gastric mucosa of fasting frogs in a dual chambered bath and exposed the inner surface to various solutions. The descending order of the sensitivity of the gastric glands to the various ions was as follows: calcium, potassium, magnesium, phosphate, and bicarbonate. An external supply of phosphate and bicarbonate ions was not necessary for the formation of acid by the glands in these preparations. Babkin, Hebb & Krueger (12) found that the introduction and retention for two to two and one half hours of 1 per cent acetic acid into the stomach of dogs with an esophagotomy and gastric fistula, greatly altered the secretory response to subsequent sham feeding with meat or subcutaneous injection of histamine. The volume of gastric juice, total chloride concentration, free and total acidities were lowered and the con-

tent of mucus increased. These workers considered the lowering of gastric acidity and total chlorides as due to the neutralizing and diluting effect of the mucoid secretion. Grant (13) placed isotonic hydrogen chloride of physiological strength in the stomach of anesthetized cats before and after the secretion of gastric mucus. Mucus secretion was excited by 1 per cent acetic acid or 80 to 95 per cent alcohol. As the acidity of the introduced hydrogen chloride was lowered, its calcium content was increased. Gray & Bucher (14) determined the composition of gastric juice from the vagotomized pouches of the entire stomach of dogs. The output of hydrogen chloride, chlorine, base chloride, potassium, sodium, and calcium increased with the rate of secretion in linear fashion but at different rates. On the other hand, the concentration in the gastric juice of base chloride, sodium, and calcium decrease and the concentration of chlorine, hydrogen chloride, and osmotic pressure increase with the rate of secretion in hyperbolic fashion but at different rates. The concentration of potassium remained constant in the gastric juice. Inasmuch as the nonparietal component is secreted at a very slow and practically constant rate and the parietal component is secreted at rates which vary widely with the dosage of histamine, the authors concluded that the composition of gastric juice is a function of its rate of secretion. In dogs with pouches of the greater curvature of the stomach, Lifson, Varco & Visscher (15) found that pilocarpine produced juice that had a lower osmotic activity and higher total nitrogen than juice stimulated by histamine. Juices under fasting conditions showed highly variable relations between total nitrogen and osmotic activity. Juice from the pyloric pouch had a higher total nitrogen than that from the greater curvature. It was hypertonic to blood and its osmotic activity calculatd from its electrolyte content was less than the observed value. After gastroscopic visualization in humans, Lerner, Asher & Andrews (16) observed that during fasting the excretion of neutral red was limited to scattered areas in the fundus and that few of the pepsin and hydrogen chloride secreting cells were functioning. Berk, Thomas & Rehfuss (17) have decided that the end points of the color indicators, dimethylaminoazobenzene and phenolphthalein, are uncertain and suggested the use of comparative buffered colorimetric standards to control the end point. The method of Hollander and Jemerin for making Pavlov pouches has been modified by Gregory, Hallenback & Code (18) to preserve more of

the vagal fibres. The secretion of five sulphonamides in the gastric juice from Heidenhain pouches of dogs was determined by Cooke, Davenport & Goodman (19). Sulfanilamide and sulfapyridine were 2.6 and 3.2 times, respectively, as concentrated in the juice as in the plasma and the others less than the plasma. The concentration ratios of the sulfonamides in juice and plasma appeared to be independent of the secretogogue and tended to vary directly with their concentration in the blood and inversely with the rate of gastric secretion.

Gray, Wieczorowski, Wells & Harris (20) have described the preparation and properties of urogastrone. It was not affected by pepsin. Intravenous injection of the preparation into dogs with vagotomized pouches of entire stomach in doses of 0.5 mg. inhibited the gastric secretory response to histamine. The extracts produced a mild inhibition of gastric motility but only if the vagi were intact. The best extracts inhibited pancreatic and biliary secretions but not salivary secretions. The apparent excretion of urogastrone was decreased by removal of the small intestine and increased by induction of diuresis, by the ingestion of high or low fat diets, and by the exclusion of digestive secretions from the small intestine (21). Friedman & Sandweiss (22) reported that the gastric depressant in urine, inhibited not only gastric secretion which had been excited by insulin, histamine, or food but also gastric motility.

Necheles & Olson (23) found that histaminase had no depressant effect on the salivary, gastric, biliary, and pancreatic secretions of dogs stimulated by histamine. It had no depressant effect on gastric secretion of dogs following a meat meal or the gastric motility which had been excited by insulin or prostigmine. Schiffrin (24) determined the effect upon the gastric glands of the administration of parathromone to dogs in amounts sufficient to elevate serum calcium approximately 2 mg. per cent. In dogs with Pavlov pouch the volume and acidity was decreased and the pepsin of gastric juice was increased in response to test meals and histamine. In dogs with a Heidenhain pouch, the volume, acidity, and chloride concentration increased without affecting the pepsin. After thyroparathyroidectomy, the volume increased and pepsin decreased in the gastric secretion of all dogs. This hypersecretion was decreased by the injection of calcium lactate or parathormone. Administration of thyroxine did not affect the gastric secretion. Lyamin & Shargorodskaga (25) reported that during acute and

chronic malaria there was a diminution of gastric acidity and tone, and delayed gastric emptying. Gordon & Chernya (26) found that, during hypnosis of patients with gastric fistula, gastric secretion stopped and that hypnotic suggestion of food caused much secretion. Hypoglycemia stimulated secretion. A symposium on the mechanism of secretion has been presented (27). Thomas (28) has devised an improved cannula for gastric and intestinal fistulas. Berk, Rehfuss & Thomas (29) have described a method for the simultaneous aspiration of gastric and duodenal contents.

Gastric motility.—Quigley and co-workers (30), in studies made with the tandem balloon method, found that the introduction of fats into the duodenum of conscious fasting dogs inhibited the motility of the pyloric antrum, sphincter, and duodenal bulb. A quantitative study of pressure relations in the pyloric sphincter region in combination with a visualization of motility and propulsion showed that cream in the duodenum inhibited the pyloric region and decreased or reversed the antral-bulbar basal and phasic pressure gradients. Fats retarded gastric evacuation chiefly by decreasing antral propulsive peristalsis. Sphincter spasm was not involved and evacuation was retarded in the presence of sphincteric relaxation. The action of cream was most pronounced in fasting animals. Using a similar technique, Quigley & Meschan (31) demonstrated that the introduction of fatty acids or soap into the proximal intestine of fasting dogs inhibited the motility of pyloric antrum, sphincter, and duodenal bulb in a manner qualitatively similar to that produced by natural fats. In producing inhibition of the sphincter region the fatty acids were from 1 to 2.5 times as effective as natural fats. Tidwell & Cameron (32) after experiments on cats, rats, and one human concluded that there was a striking parallelism between the ease of absorption of a fat and its ability to produce gastric inhibition. Thomas (33) introduced separately sixteen amino acids into a fistula of the small intestine of dogs. Only monoamino, monocarboxy acids caused gastric inhibition regularly in neutral solution. The dicarboxy and diamino acids caused gastric inhibition when administered as free acids without neutralization but were ineffective in neutral solution. Van Liere & Northup (34) decided that gastric emptying in men was not influenced by senescence. The average normal emptying time of twelve men whose ages ranged from 58 to 84 was 1.94 hours with extremes of 1.33 to 2.75 hours. In fifty-nine young adults the aver-

age emptying time was 2.08, with extremes of 1.03 to 3.08 hours. In ten normal young adult humans, a dose of 0.2 gm. of sodium amytal decreased gastric emptying time on the average of 19.7 per cent. The decrease was statistically significant in nine of ten subjects (35). Folley & Abbott (36), using the intubation technique in normal human subjects, found that their gastric emptying curve was not affected by certain commonly used drugs with the exception that morphine produced a delayed inhibition. Auer & Krueger (37) reported that the gastric and cecal peristalsis excited by drugs in barbitalized rabbits was inhibited by inhalations of a half and half mixture of oxygen and carbon dioxide. In case of the cecum the inhibition was peripheral. Necheles, Olson & Morris (38) reported that the subcutaneous injection of 0.65 units of insulin per kg. of body weight into dogs was followed in most instances by a prolonged depression of gastric motility and tone. This depression was coincidental with low blood sugar and, as blood sugar rose to medium or slightly subnormal value, the typical insulin hypermotility appeared. Pierce, Haege & Fenton (39) found that a solution of 50 per cent glucose, when introduced into the stomach of rats anesthetized with pentobarbital, was diluted rapidly by the gastric residuum and secretion. The average concentration of glucose in the gastric contents after one hour is approximately 20 per cent regardless of whether 650 or 1000 mg. of glucose are introduced as a 50 per cent solution. Gibb (40) has reported some observations with a gastroscope in clinical cases. The pylorus he found to be open practically all the time and to close only for a brief interval when the contraction wave from the antrum reaches and blends with it. He described a new structure, the musculus sphincter antri, as a rope like ridge located upon the greater curvature at right angles to the long axis of the stomach, just opposite the incisura and extended about halfway upward on the anterior and posterior wall. Lehmann & Knoefel (41) have studied the effect of atropine and related substances upon gastric motility in dogs. Tudoranu & Dimitriu (42) have reported that the introduction of vitamins A or B_1 into the human duodenum produced within a few minutes an increased motility of the stomach. Vitamin C and vitamin D inhibited gastric movements.

Intestinal contents.—McGee & Hastings (43) found that normal human jejunal juice, collected by means of a Miller-Abbott tube, tended to have a characteristic acid-base balance with a bicarbo-

nate concentration about one third that of the blood serum (8 m*M* per liter) and a carbon dioxide tension about double that of venous blood (100 mm.) resulting in a pH of 6.5. Displacement of the normal acid-base balance by acid or alkaline salts or by dilution resulted in a prompt return of the acid-base balance to normal. Berk, Thomas & Rehfuss (44) found in normal conscious dogs that after an Ewald meal the pH of the duodenal contents just below the pylorus was consistently greater than the gastric contents just above the pylorus, the difference amounting to about three units. The contents of the first part of the duodenum in the normal dog displayed a neutralizing, buffering, and diluting capacity in excess of physiologic requirements so that free acid although not always absent, was usually missing. Variations in gastric free acid were not regularly accompanied by changes in duodenal pH. Hence, gastric acidity is probably not a reliable index of the effective acidity of the contents of the duodenum which is the region with the greatest incidence of ulcers in clinical cases. Following a meal consisting chiefly of carbohydrate, the acidity of intestinal contents was rarely sufficient to affect gastric motility significantly but might be a factor in stimulating pancreatic secretion. Fat in the form of cream effectively diminished the acidity and enhanced the excess neutralizing ability of the contents of the first part of the duodenum over fairly prolonged periods of time in the normal dog (45). The decrease in duodenal acidity following cream was only partly due to the inhibitory effect of fat on gastric secretion. The effective acidity of the duodenal contents was found to have no parallel relationship with any of the customary measures of gastric acidity in the normal dog and was largely determined by the type of food undergoing digestion and only partially by the acidity of gastric contents.

In fasting dogs, the neutralizing capacity of the contents in the first part of the duodenum was equal to and in some respects exceeded that of the digestive state. In addition, there was the tendency for the various criteria of acidity in the stomach and duodenum to show wide fluctuation but to aggregate constancy. The average pH in the stomach was 1.90 and in duodenal bulb, 5.56 (46).

Supplemental amylase in taka-diastase had no effect on starch digestion in humans with the average mixed meal unless a salivary deficiency occurred according to the studies of Beazell (47). With

a finely divided meal it augmented starch digestion. Taka-diastase had no effect on protein digestion or gastric emptying. In one patient with an ileostomy it had no effect on the amount of nitrogen or starch lost. In dogs with duodenal fistula, the average starch digestion was 7.5 per cent and with taka-diastase, 38.9 per cent.

Davison (48) has pointed out the difficulties in the enzymic titration of duodenal contents for trypsin and amylase. He (49) also found that gastric contents increased several times the action of amylase. Anderson (50) has reported that the assay of trypsin in duodenal contents was a reliable means of diagnosis of pancreatic deficiency. Assays for amylase and lipase were considered less reliable. Kolm, Shay & Gershon-Cohen (51) have described a method of determining trypsin in the duodenal contents with the Evelyn colorimeter.

In roentgenograms, Paine & Nessa (52) observed in children up to eighteen months in age that gas was distributed about equally between small bowel and colon. Between eighteen months and six years more gas is found in colon and less in small bowel and after seven years of age, as in adults, no gas appeared in the small bowel. In seven newborns, gas was found in the stomach, duodenum, and jejunum within six to fifteen minutes after birth.

In order to test the suggestion that lymphocytes are lost from the blood through the intestinal tract, Yoffey (53) removed the jejunum, ileum, most of the large intestine, and the glandula mesenteric magna from dogs. In these animals, he found no constant change in the count of blood lymphocytes within two to three hours after operation.

Intestinal motility.—Grindlay & Mann (54) studied the motility in trained dogs of exteriorized loops of small intestine, prepared by a modification of the Biebl loop technique. In dogs fasted for forty-eight hours the loops were usually quiet. The introduction of a stomach tube in sham feeding or the introduction of water or various liquid meals and the feeding of a solid cooked meal caused a typical motor response which was maintained only in case of a solid meal, when it was sustained for hours. Ackerman, Curl & Crandall (55) found that dogs with a bile fistula, when given a mixed meal, exhibited an increase in rate of gastric emptying and of entrance of barium into the large intestine. After a fat meal the rate of gastric emptying in these dogs was slower than in normal dogs, possibly because of the formation of increased amounts of

enterogastrone. Administration of bile salts with the fat meal brings the gastric emptying time to the values for a normal dog.

Ingelfinger & Moss (56) intubated twenty human subjects with a double lumen tube which connected to tandem balloons and induced nausea by caloric stimulation of the labyrinth or by morphine. In thirteen of eighteen attacks of nausea, there was a generalized contraction of the descending duodenum which often expelled both balloons backward into the stomach, although no reverse peristalsis was observed. Therefore, the passage of duodenal contents into the stomach is caused by a reversed pressure gradient.

Youmans, Karstens & Aumann (57) used trained dogs with two high jejunal fistulae to study the nervous pathways for reflex regulation of intestinal pressure. The immediate nervous inhibition of one intestinal segment following the sudden production of a pressure of 40 or more mm. Hg in the other segment was abolished by sympathectomy. The reflex was not noticeably altered by vagotomy. It was not mediated through the decentralized celiac ganglia or through other preaortic ganglia in unanesthetized dogs even though nervous connections between these ganglia and the intestine were intact. A powerful contractile response might be induced by sudden distention of the intestine after sympathetic denervation. This type of motor response was not produced in normally innervated intestine because this inhibitory reflex involves the site of distention as well as the undistended intestine above and below the distention.

Elevation of metabolism by 50 per cent with thyroid extract or by 200 to 500 per cent with 2,4-dinitrophenol did not increase the rate of contraction of exteriorized intestinal loops in dogs except in a segment just above the iliocecal valve according to Oppenheimer & Glyer (58). Castleton & Alvarez (59) found that experimental hyperthyroidisim in the rabbit resulted in an increased rate of rhythmic contraction of the excised small intestine and of the intact intestine in the anesthetized animal.

Short wave diathermy did not produce any change in motility of exteriorized intestinal loop in dogs except that due to direct application of heat to the bowel wall. McLoughlin, Mann & Krusen (60) found that the only change was a slight increase in rate of rhythmic contraction. Bisgard, Matson & Hirschman (61) observed that heat applied to the abdominal wall of humans inhibited the motor activity of stomach, small bowel, and colon.

Cold applications stimulated tone and peristalsis. These responses were reversed when the patient drank hot or cold fluids.

Kozoll & Necheles (62) found that ileocolostomy in the dog was followed by a two hour decrease in the time of colonic filling. Following resection of the colon proximal to the anastomosis, this time was further shortened. Retrograde filling of the proximal excluded colon was an invariable accompaniment of ileocolostomy in dogs. Consequently, in this condition it cannot be assumed that this method gives physiologic rest to the part. Wasteneys, Crocker & Hamilton (63) have described the preparation and care of dogs with a re-entrant intestinal fistula. The emptying of the small intestine after a haddock and barium meal was determined by the x-ray and by collection of the contents at the fistula. The two methods with this meal gave the same result but with a meal of beef heart and barium the collection of material was only 70 per cent. In this case barium passage was unreliable. Cellulose seemed to pass more slowly than protein. Pork fat and lamb fat increased the time for emptying a haddock meal threefold and beef heart fat doubled it. Alles (64) has reported studies of the action of certain carbon-ammonium salts upon the motility of isolated intestine. Cantor (65) ties a bead on to the end of a duodenal tube in order to facilitate the tube's passage into the duodenum.

Intestinal absorption.—The rate of absorption of glucose from the rat's intestine under forced feeding with strong glucose solutions depended upon the amount and concentration of the sugar according to MacKay & Clark (66). Under normal or at least voluntary feeding conditions, absorption rates several times higher than those previously reported were found. By concentrating the period of eating through a preceding fasting period and offering 95 per cent glucose, absorption coefficients of 300 mg. were obtained. By giving glucose alone and stimulating appetite with protamine zinc insulin absorption was increased to 200 to 300 mg. A cold environment and 50 per cent glucose gave absorption exceeding 500 mg. Hamar (67) found that normal rats fasted for four days had a greatly reduced rate of glucose absorption and that this might account for two thirds of the impaired glucose absorption which occurs after adrenalectomy. Further, he reported that dissolving the glucose in the intestinal content from a normal rat resulted in greater absorption by the adrenalectomized rat than when the glucose was given in Ringer's solution. He (68) also found the rate of

glucose absorption in the rat was lowest at 9:00 a.m. after which there was a practically linear increase in absorption until about 5:00 p.m. Sciclounoff & Alphonse (69) reported that lactoflavin given parenterally in one patient improved glucose absorption. Sinclair & Fassina (70) reported that rats which had been raised from weaning on a high carbohydrate, low fat diet and which were suffering from a deficiency of essential unsaturated fatty acids absorbed 1.5 times more glucose per 100 gm. of body weight than the controls. Mature rats fed the same diet for three weeks showed no change in glucose absorption but if fed a high fat diet showed a pronounced decrease in rate of glucose absorption. In anesthetized dogs, Northup & Van Liere (71) found that anoxia up to and including a partial pressure of oxygen of 53 mm. Hg did not alter glucose absorption but at this anoxic level glycine absorption was depressed. According to Rafferty & MacLachlan (72) following the administration of starch or glucose to rats kept at about 36° the liver glycogen was lower and blood sugar higher than the controls kept at 21°. Abreu & Emerson (73) found no difference in the absorption of lactose and beta lactose in barbitalized cats and rats.

Winter & Crandall (74) using the angiostomy technique in normal unanesthetized dogs could obtain no evidence of the portal absorption of fatty acids during fat absorption. By means of spectroscopically distinguishable conjugated fatty acids of corn oil Barnes, Miller & Burr (75) could find no apparent parallelism between the rate at which the fatty acids were incorporated into the mucosal phospholipids and the mucosal neutral fat. Therefore, if phosphorylation is an integral part of fat absorption, the synthesis of neutral fat from phospholipid must be occurring at an extremely high rate, permitting only traces of the latter to accumulate. These workers (76) also reported that the amount of fat absorbed in eight hours by rats deficient in essential fatty acids is less than that of rats cured of the deficiency but if absorption is calculated on a weight basis there is no significant difference. In the deficient rats, there was a decrease in the incorporation of the labeled fatty acids into the phospholipids of the intestinal mucosa. The rates of incorporation of tagged fatty acids into the mucosal neutral fat and phospholipids were very similar for the normal and adrenalectomized rats (77). On the other hand, Bavetta, Hallman, Deuel & Greeley (78) reported that after adrenalectomy in rats fat absorption was inhibited and larger amounts of fatty acids ac-

cumulated in the intestine. Both changes were restored to normal by administration of cortin. Reiser (79) determined the lipids in the mucosa of the first 100 cm. of the small intestine of swine during fat absorption. He found no change in phospholipid or cholesterol content and only a slight amount of triglycerides during fat absorption. About 2.5 per cent of the dried weight of the intestinal mucosa was free fatty acids which amount was doubled five hours after ingestion of oil. These observations are at variance with those of other workers on other species. Since Reiser seemed to find in the mucosa during absorption more fat histologically than chemically, he suggested that there might be an uncovering of structural lipoids during this process. Frazer, Stewart & Schulman (80) found that rats would absorb 60 per cent of an administered dose of paraffin oil which had been emulsified to a droplet size of 0.5μ. Stryker (81) found liquid petrolatum to be absorbed in small amounts from the intestine of rabbits, rats, guinea pigs, and human subjects. The oil is most readily demonstrable in mesenteric lymph nodes but may be present in intestinal mucosa, liver, and spleen.

Smith & Crandall (82) found that dogs with a bile fistula which had been maintained for long periods without oral administration of bile remained persistently anemic and yet they absorb iron, as judged by the rise in plasma iron, in a normal way if it is given alone or with their standard diet. Iron given with neutral fat is absorbed so slowly by the bile fistula dogs that no change in plasma level occurs. Peters (83) found in barbitalized dogs that bile salts decreased water and active chloride absorption. He (84) also found that, in loops of lower ileum in anesthetized dogs, chloride accumulates very slowly in originally chloride free solutions. The highest concentration observed was 0.032 per cent sodium chloride. Stevens, Taliaferro & Hoag (85) found no significant difference in the absorption of sulfathiazole through the intestine, pleura, or peritoneum of dogs. Less than 10 per cent, if any, of galacturonic acid is absorbed from the canine small and large intestine and human small intestine according to Werch & Ivy (86). It did stimulate the secretion of intestinal fluid in the dog and human. Driver (87) reported that lowering surface tension would not promote intestinal absorption of glucose in the rat. Hexylresorcinol and ammonium thiocyanate inhibited the absorption of chloride from the dog's ileum. Inasmuch as these compounds inhibit carbonic anhydrase, Driver suggested a role of this enzyme in the absorption of chloride.

Hart & Essex (88) studied water metabolism of the chicken with reference to the role of the cloaca. After creation of artificial anuses or exteriorization of ureters, some birds had a temporarily increased water intake. Addition of salt to the diet increased water intake and its omission resulted in pronounced weight loss and death with extreme depletion of body water. Fistulous birds lost weight more rapidly than controls when drinking water was withheld indicating that normally the rectum absorbs water. Nicholson & Chornock (89) have described an improved intubation technique for studying intestinal absorption in humans. They found that the capacity of a short segment of the upper small intestine to absorb ascorbic acid within one hour far exceeded the optimal daily requirement. Other aspects of intubation have been reported (90). Rockwell (91) has demonstrated that after the oral ingestion of pollen by rabbits, the active pollen antigen appears in the blood, kidneys, liver, and muscles. Extracts of the rabbit's tissues were made and tested on patients sensitive to ragweed. Harten, Gray, Livingston & Walzer (92) have reported that in anesthetized monkeys whose esophagus or stomach had been surgically sectioned between clamps, there was absorption of unaltered protein from raw ground cottonseed through the esophagus or through the stomach.

Colon.—Adler, Atkinson & Ivy (93) have studied colonic motility in four healthy males, in whom a colostomy had been established. Three types of movement have been described. Reversed movements in the descending colon of their subjects were rare. Sleep tended to depress and awakening to augment colonic motility. Garrer, Groen & Hallen (94) have reported that, in four healthy men, administration of 5.8 to 46.4 gm. of glucose in isotonic solution by rectum is not followed by a rise in blood sugar.

Pancreas.—Harper & Voss (95) studied the control of the external secretion of the pancreas in cats that were anesthetized or decerebrated. Secretin was repeatedly injected intravenously every ten to fifteen minutes. These workers considered all secretory fibres to the pancreas to be contained in the dorsal vagus trunk. The splanchnics contained inhibitory fibres to the pancreatic acini. Central vagal or sympathetic stimulation was either without effect or caused inhibition. Stimulation of the peripheral vagus had no effect upon the rate of secretion. The presence of foodstuffs in the stomach did not stimulate the pancreas. However, the passage of food, inulin, normal saline, or water through the pylorus into

duodenum or their injection into the duodenum resulted in an increase in enzyme output and in most cases an increased volume. The increase in enzyme output could be observed after section of all extrinsic nerves to the small intestine and after acid and bile were excluded from the intestine. Distention of the duodenum with a balloon had no effect upon either enzyme output or juice volume. Secretin did not increase the production of enzymes by the pancreas.

Barrington (96) repeatedly injected secretin preparations at intervals of five to fifteen minutes into anesthetized cats which were either atropinized or vagotomized. He found that enzymes continued to be discharged in the pancreatic juice for more than six hours. A continued decrease in output of enzymes did not necessarily occur in his experiments. If the rate of flow of pancreatic juice was increased either by administration of sodium nitrite or by accelerating the rate of secretin injection, only the latter resulted in an increased concentration and output of enzymes. Consequently, he concluded that secretin directly stimulates the secretion of enzymes. In conscious dogs, Thomas & Crider (97) found that the products of protein digestion acted in the intestine as stimuli for the secretion of pancreatic juice. Their effectiveness was not dependent on the coincident flow of bile into the intestine nor on the secretogogue action of water or acid. Secretion produced by peptone stimulation had a higher specific gravity and contained a greater concentration of nitrogen than that produced by water, acid, or secretin. Properties of this secretion were such that it could not be produced by secretin alone but it did resemble that caused by pilocarpine, suggesting that peptone acts through a nervous mechanism. By the use of a two-lumen duodenal tube in human subjects, Comfort & Osterberg (98) found that mecholyl chloride appeared to increase those components of the duodenal contents which probably came from the pancreas more uniformly and more potently than did casein, fat, or prostigmine methyl sulfate. Secretin was more effective than the above and it plus mecholyl chloride produced the greatest secretion of the pancreatic components of the duodenal contents. Using a similar method, Pollard, Miller & Brewer (99) determined the pancreatic response of clinical patients to an intravenous injection of secretin. There was diminished secretion if a carcinoma were in the head but not in other parts of the pancreas; no marked association of disturbed

pancreatic secretion and either biliary disease or in one case, chronic pancreatitis. However, four of six cases of severe diabetes mellitus had distinctly low volumes of secretion plus low concentration and total output of bicarbonate. In one case there was low amylase and in another low amylase and high trypsin. Necheles, Plotke & Meyer (100) compared the pancreatic secretion of a group of thirty humans with an average age of 66.5 years with one of 23.4 years. In the secretion stimulated by oleic acid, the sodium bicarbonate and amylase were increased and lipase 21 per cent lower in the older group. There was no difference in volume of fluid and trypsin. Greengard, Roback & Ivy (101) reported that certain sympathomimetic amines inhibited and others stimulated pancreatic secretion in dogs. Sproul & Sanders (102) found that deprivation of the external pancreatic secretion in cats by section of ducts or pancreatectomy was followed by an increase in lipid content of the stool. The average prothrombin time was increased and vitamin K of the liver decreased, which effects were more striking after pancreatectomy. There was only moderate alteration of blood clotting and slight prolongation of bleeding time. Ball, Tucker, Solomon & Vennesland (103) intravenously injected into dogs bicarbonate formed from radioactive carbon. It promptly appeared in the pancreatic juice in concentrations four to five times that of serum. Since the total carbon dioxide of the juice was about five times that of the serum it was concluded that the plasma, rather than metabolic processes, was the chief source of the carbon dioxide in the juice. Tucker & Ball (104) found that injection of sulfanilamide into dogs in doses sufficient to result in a concentration of sulfanilamide in the pancreatic juice two hundred times that necessary to inhibit carbonic anhydrase was without effect on the rate or composition of the juice. Greengard, Stein & Ivy (105) in assays of human urine demonstrated the presence of the enzyme, secretinase, and a thermostable principle which directly inhibits the secretory activity of the pancreas. According to Scott, Collignon, Bugel & Johnson (106), insulin hypoglycemia increased the volume of pancreatic juice secreted by unanesthetized dogs with permanent pancreatic fistulae. Bilateral vagotomy or exclusion of gastric juice from the duodenum by pyloric section abolished this effect.

Montgomery (107) has reported that successful ligation of pancreatic ducts in dogs led to atrophy of pancreatic acini and to al-

terations in the blood and liver lipoids that were apparently comparable with the blood and liver lipoid changes in a depancreatized dog maintained with insulin. Unactivated pancreatic juice fed to depancreatized dogs maintained with insulin or to dogs with ducts ligated controlled blood and liver lipoids in a manner indistinguishable from that which follows administration of raw pancreas. Vermeulen, Dragstedt, Clark, Julian & Allen (108) found that the simultaneous oral administration of lipocaic and cholesterol to rabbits did not prevent the hypercholesteremia and arteriosclerosis which was due to the cholesterol. Beling, Baker & Marquis (109) by taking roentgenograms of two patients with disseminated calcification of the pancreas found the pancreas a very mobile organ in the abdomen as the subject changed position.

Nutrition and the digestive system.—Dyer & Roe (110) have reported that acute or chronic vitamin A deficiency had no effect on the secretion of acid or mucin in the rat's stomach. Stryker & Janota (111) could detect no effect of vitamin A deficiency in rats upon the permeability of their digestive system to the toxins from *S. enteritidis* or *Cl. botulinum*. Russell & Nasset (112) used dogs with a Maydl jejunostomy 50 to 75 cm. from the ligament of Treitz. Fresh yeast had a distinctly stimulating effect on gastrointestinal motility which seemed to be largely local. The increased motility was often but not always accompanied by increased rates of digestion and absorption of carbohydrate. Dried yeast plus a 50 per cent alcoholic extract of fresh yeast had no effect on motility but did increase the rates of digestion and absorption by 20 per cent. Dyer & Roe (113) found in rats that acute or chronic B_1 deficiency had no effect upon the duration of secretion during mecholyl stimulation, upon the concentration of total or free HCl, amount of mucin, or peptic activity. Gershon-Cohen, Shay & Fels (114) found in roentgenograms of vitamin B_1 deficient rats gastrointestinal hypotonicity, dilatation, and stasis. Heublein, Thompson & Scully (115) made some studies of the effect of a deficiency in the vitamin B complex upon gastric emptying and motility of intestine in the dog. There was a drop in food intake and body weight which was partially corrected for five to fifteen days by giving B_1. Nicotinic acid and lactoflavin had little effect. There was marked impairment in gastric motility and some loss in the intestine. Martin, Thompson & Forero (116) found that administration of inositol to dogs receiving 10 per cent yeast in their diet markedly increase the peristalsis of stomach and small intestine. Thiamine, riboflavin,

choline, pyridoxine, and pantothenic acid did not produce this effect. Etzel (117) has suggested that megaesophagus and megacolon might have an etiologic basis in chronic B_1 deficiency. Roe, Hall & Dyer (118) found in the guinea pig a marked reduction first in volume and later in acidity of the gastric juice when the stage of advanced scurvy occurs. This was not due to inanition.

Beams, Free & Glenn (119) found evidence of impaired absorption of galactose in all patients with active pellagra and nontropical sprue. Improvement in absorption accompanied clinical improvement in pellagra but not in sprue. Approximately half the patients with pernicious anemia showed impaired absorption which did not parallel changes in red cell count.

Pathologic Physiology

Bowel obstruction.—Antoncic & Lawson (120) found the intestinal pressure in closed jejunal loops of unanesthetized dogs to range from 33 cm. of water to 16 cm. with an average of 26.8 cm. Contractility was greater in obstructed loops and neither it nor the pressure, nor irritability to epinephrine declined at the end of survival. Lawson (121) found increased arterial flow into loops of ileum in dogs following a period of intestinal distention. Since it was associated with augmented venous flow and was greater in volume than the demonstrable volume reduction of the bowel wall he concluded that less than 25 per cent of the deflation hyperemia could be due to filling of the vascular bed whose pressure was temporarily lowered by the decompression. The rest of the excess blood flow during deflation could be abolished by local application of cocaine and it failed to increase in magnitude with increasing duration of distention. He considered this part of the hyperemia to be due to the persistence of a resistance lowering mechanism in the bowel wall. Lawson & Ambrose (122) found in anesthetized dogs that during moderate distention of the ileum there was a marked rise in the oxygen content of venous blood returning from the loop. After cocainization of the intestine, the oxygen content of the mesenteric venous blood was always reduced upon distention of the bowel. These observations suggested that distention caused the opening of low resistant vascular shunts in the bowel wall. Gatch & Battersby (123), on the basis of studies on anesthetized dogs, concluded that distending pressures to the intestine of less than half diastolic blood pressure did little injury to the bowel unless they acted continuously for a long time. Distention great enough

to injure the bowel exerted its effect in two stages. At first, the bowel was tense and anemic and later flaccid and congested. It passed from the first to second stage when the wall stretched enough to permit resumption of blood flow in fair volume. Paine, Lynn & Keys (124), two weeks after making a Biebl loop of the terminal ileum in dogs, produced obstruction below the loop. Respiration of 95 to 100 per cent oxygen by these increased the oxygen concentration in the obstructed bowel from 5.8 to 22 per cent and decreased the carbon dioxide from 15 to 2 per cent for twenty-four to thirty-six hours. After this time oxygen decreased and carbon dioxide increased. Neuwelt, Medoff, Patedjl & Necheles (125) found that following pyloric obstruction and gastroenterostomy in dogs, the volume and acidity of the gastric juice was not consistently changed and gastric emptying time was unaffected. Hill, O'Loughlin & Stoner (126) ligated a loop of dog's jejunum, including the mesenteric vessels, but did not completely obstruct the arterial flow. Within one hour the peritoneal fluid contained many red and white blood cells and in two hours many cells as well as short chained streptococcus and gram negative short rods. Childs & Scudder (127) found, following twenty-four hours of duodenal distention in rats, that there was an elevation of whole blood potassium and specific gravity, which values returned toward normal within one hour after release of the distention. However, after forty-eight hours of distention with higher pressure, the whole blood potassium and specific gravity abruptly increase when the distention was released. In the latter case, the bowel was gangrenous. Hamilton & Curtis (128) observed in clinical patients that in early pyloric stenosis gastric hyperactivity was associated with clinical evidence of pylorospasm. In long standing pyloric stenosis, when the stomach had become dilated and decompensated, it remained quiescent for a long postoperative period before compensation was eventually regained.

Pernicious anemia.—In an attempt to produce this condition experimentally, Petri, Norgaard & Jensenius (129) subjected pups and adult dogs to different types of operations which consisted of removal of all or part of the stomach and duodenum. In none of the dogs did pernicious anemia develop but in all dogs a sub-pellagrous picture appeared, which was much more severe in the pups. Considerable detail is presented. Petri, Jensenius & Thyssen (130) surgically removed the pylorus, the Brunner gland section of the duodenum, and the distal two thirds of the small intestine

in pups. Pernicious anemia did not develop. There appeared a picture, characterized by arrested growth, emaciation, changes in skin and hair, degeneration of central nervous system, anemia, achylia, and periodical diarrhea. Iura (131) produced anemia, not macrocytic, in dogs by resection of the intestines and amputation of the hind legs. Nielson (132) reported four cases of pernicious anemia with gastric acidity preserved. Hernberg (133) has found that the volume of gastric juice secreted after histamine in cases of pernicious bothriocephalus anemia was about one third of the normal person. In amounts of 600 cc. this juice exerted an antianemic effect in cases of pernicious anemia. Gordon & Japa (134) have reported the development of macrocytic anemia in two elderly men after simple gastroenterostomy for ulcer. Although desiccated preparations of the pylorus of hog's stomach are effective in curing Addisonian pernicious anemia and those of the fundus ineffective, Fox & Castle (135), in a similar comparison with preparations of the human stomach, demonstrated that only those of the fundus were effective. The fundic glands probably secrete the intrinsic factor. These are degenerated in pathological examination of cases with pernicious anemia.

Stasney & Burns (136) found that immature opossums living in the maternal pouch showed a macrocytic anemia as compared with the adults. Direct injection of either concentrated normal human gastric juice or liver extract changed the blood picture to normal.

Peptic ulcer.—The physiological and medical problems involved in this condition have been extensively reviewed (137). Schiffrin & Warren (138), in intestinal perfusion experiments, found pepsin and acid more effective in causing ulceration than acid alone. Sandweiss & Friedman (139) found that the beneficial effect of urine extracts on Mann-Williamson ulcers was not due to a gastric secretory depressant. Shoch & Fogelson (140) found that feeding predigested protein prolonged survival and delayed the appearance of this type of ulcer. Aluminum hydroxide, aluminum phosphate, magnesium trisilicate, and mucin inhibited peptic activity by altering the pH of the medium, the degree of inhibition being directly related to the pH change. Sodium lauryl sulfate alone completely inhibited peptic activity without any alteration in pH (141). Eichorn (142) reported that immediate liberal feeding of patients with bleeding peptic ulcer gave more favorable results than starvation. Chum, Harkins & Boals (143) found that

administration of whole blood or red blood cells to dogs through a jejunal fistula produced alimentary azotemia although less than by intragastric administration. Whole blood introduced into the peritoneal cavity produced no azotemia. Black (144) has reviewed the azotemia in gastroduodenal hemorrhage. Schiff, Stevens, Shapiro & Goodman (145) concluded from experimental studies on man that the passage of a tarry stool did not necessarily indicate a severe hemorrhage into the digestive tract and that the persistence of tarry stools or occult blood did not necessarily indicate continuation of such hemorrhage.

Miscellaneous.—Meyer & Necheles (146) reported that prostigmine benefited two patients with achalasia and dilatation of the esophagus. Voegt (147) reported that gastric acidity was reduced in leukemia. Brown, Pendergrass & Burdick (148) concluded that in clinical hyperthyroidism increased incidence of achlorhydria, delayed gastric emptying, and increased intestinal tone and motility were some of the characteristic changes. Necheles & Olson (149) concluded that mechanical trauma in anesthetized dogs had no effects on gastric secretion and slight effects on gastric motility.

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BLOOD

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Space limitations have necessitated the exclusion of certain phases of this subject notwithstanding the sharply decreased availability of continental European literature. To those whose work has not been cited and especially to those presenting data on normal values, the reviewer extends his apologies. With some exceptions the data was published between July 1, 1941 and June 30, 1942.

Several books and monographs on hematology and related subjects have been published (1 to 6). The more clinical aspects of blood are extensively reviewed elsewhere (7).

Plasma

Plasma volume and protein.—By comparing the data on blood volumes calculated by different techniques, the conclusion is reached that in the dog the jugular hematocrit is not representative of the cell/plasma ratio of the entire blood volume (8). The plasma volume is regarded as being partitioned into a "rapidly circulating" portion and a sluggishly moving portion equaling approximately 80 per cent and 20 per cent, respectively, of the total plasma volume measured by dye dilution. A theoretical analysis places the slow moving 20 per cent in contact with the vessel walls, this fluid sheath surrounds the axial stream. Rearrangements of the vascular bed, therefore, must influence calculated blood volume. The same conclusion is derived from an independent approach (9). It follows, therefore, that conclusions based on calculations involving hemoglobin and hematocrit are open to question. The above may not be true of man (10), but it induces skepticism of plasma values deduced from cell volume measurements.

Both normal and splenectomized men respond to epinephrine and exercise in a similar manner (11, 12, 13); the total plasma volume decreases, at the same time becoming more concentrated. The experiments fail to supply evidence of reservoirs of relatively idle blood.

In man and dogs recovering from an acute hemorrhage (14, 15), the restoration of plasma volume is related to the rate of mobilization of plasma protein. The data argue that little if any preformed plasma protein exists in the body. Further, plasma added to the blood stream of normal men seems to disappear rather quickly (16, 17), and the protein may be used for nutrition in certain conditions (18). A lecture on the plasma proteins emphasizes the concept that the plasma proteins normally are in dynamic equilibrium with the cellular protein, especially that of certain cells such as the liver (19). Irrespective of whether the rate of incorporation of deuterium is a true measure of the rate of protein synthesis, it is significant that liver and plasma proteins incorporate it at the same high rate (20); the incorporation by hemoglobin is slow. Although limited by several assumptions, experiments on dogs during protein starvation and replenishment suggest that the serum albumin remains a constant fraction of the total protein level of the body (21).

Reports too numerous to cite are concerned with the production techniques, utility, and value of preserved plasma although there is controversy over the relative merits of pooled and unpooled serum versus plasma, and normal versus four normal plasma. Of the several plasma substitutes suggested, pectin (22, 23, 24) and animal albumin (25) appear most promising. The difficulty with serum albumin lies in freeing the albumin fraction of globulin (26, 27).

An address on the subject of antibodies and protein reserves (28) must be read in the original.

Coagulation.—Only a few findings need be added to the previous extensive review (29). Chloroform treatment of serum yields a material associated with euglobulin, which possesses thromboplastic, fibrinogenolytic, and fibrinolytic properties and in many respects resembles trypsin (30). It is suggested that contamination of coagulating solutions by tryptase is responsible for data leading to the view that fibrin formation is a denaturation process. With sufficiently pure preparations no evidence of a double flocculation maximum for fibrinogen is found (31). Since blood platelet extract possesses tryptic activity (32), the platelets may be the source of the contamination. The antitryptic activity of serum decreases upon initiation of shock in guinea pigs, but during the reaction the antitryptic power increases; surviving animals have

high antitryptic activity (33). (See also sections on heparin and platelets.)

A pseudoglobulin fraction prepared from rabbit plasma (34 to 38) possesses extraordinary thrombic activity, being active by the parenteral route and by the enteral route if it escapes gastric digestion.

The passage of a weak direct current through blood inhibits the coagulation process, and in addition the electrode metal relative to direction of current influences specifically the deposition of fibrin; the reasons for the phenomena are not clear (39). The anticoagulant action of sodium polyanetholesulfonate, "Liquoid," is related to an undesirable precipitating effect on calcium ions with protein (40). Clot retraction is stated to depend on mesh size of the fibrin net, which in turn is related to the velocity of coagulation (41).

An important contribution to the understanding of the interrelations of coagulation and capillary activity records the results of microscopic observations on man. Severed skin vessels successively dilate, contract, and dilate; the clot forms during the contraction phase and normally becomes securely fixed. Defective coagulation results in dislodgment of the clot when the vessel dilates. In purpuras not associated with thrombocytopoenia, on the other hand, the vessels appear to react abnormally. The discussion provides a clear concept of the necessary synergistic mechanisms (42).

Prothrombin and vitamin K.—There exists a tendency to regard estimates of the first stage of coagulation or prothrombin time as synonymous with prothrombin concentration; it is reasonable to do so only when all inhibitor systems are rigidly controlled and prothrombin is the limiting factor. The extent to which anticoagulant systems interfere with clinical assays is not assessed, and the possible inhibitors (43, 44, 45) can not be dismissed in view of anomalous data reported (46, 47). Investigations of the normal by a given technique reveal individual and daily variations (48, 49). Loss of plasma carbon dioxide (50, 51) and pH shifts to the alkaline side (52) lengthen the prothrombin time and provide another source of variation.

The method employed influences the data obtained. Studies on the same patients by the single and two stage methods afford both quantitative and trend differences (53). The present lack of agree-

ment on the value of the prothrombin time-vitamin K response in the differential diagnosis of liver disease (54 to 59) may be the result of the techniques of assay used (53). The prothrombin "concentration" during pregnancy is reported to be increased as much as 300 per cent above normal according to one method (60), but not by another (61). The utility of lecithin and Russell's viper venom as a standarized thromboplastic agent is proposed (62), but the plasma dilution versus time curve is even more sharply asymptotic than that reported for animal thromboplastin (63); this is disadvantageous and hints at a different activation process. The use of venom without lecithin is suggested (64).

The administration of an excess of a K vitamin to normal persons results in an insignificant decrease of prothrombin time (65). Vitamin K appears not to be the limiting factor in the process of prothrombin synthesis in the normal; occasional prothrombin times shorter than normal, therefore, are of uncertain significance (46, 47, 57). Nothing definite is known of the mechanism by which vitamin K is related to prothrombin, but the evidence (66) favors the view that it is required at some stage or stages of prothrombin synthesis in the liver. Exposure of an animal to x-rays to the point of marrow aplasia affects the prothrombin time only when the liver is not shielded (67).

According to reports, divergent possibly due to the criteria of hemorrhage employed, vitamin K therapy influences neonatal hemorrhage favorably (68) or not at all (69). In liver disease the bleeding tendency can not be attributed solely to prolonged prothrombin times (70). An examination of many reports does not yield a zone of prothrombin times within which spontaneous hemorrhage invariably occurs.

The K vitamins possess irritative (71) and toxic effects attributable to their redox properties (72, 73).

A standard bioassay method for K activity is proposed using the 2-methyl-1, 4-naphthoquinone structure as the standard (74).

Heparin.—Since the assayed anticoagulant activity of crystalline heparin prepared from varous species differs markedly (75) and varies with chemical treatment (76), it appears that bioassay methods are obligatory at present. Because heparin acts on both stages of the complex coagulation system (77), estimates of activity by any bioassay technique (76, 78, 79) must be considered in the light of the method employed. However, the differing anti-

coagulant potencies *in vitro* of heparin from the various species disappear *in vivo* in proportion to the *in vitro* unitage (75). The views of the two laboratories most active in the study of heparin differ sharply in a number of respects (75, 80, 81). Heparin apparently is established in the surgical armamentarium.

It is reasonably well proved that the prolongation of coagulation time of blood of dogs in anaphylactic shock is due to the liberation of heparin from the liver (82).

Dicoumarin.—3,3′-methylene-bis-(4-hydroxycoumarin), "dicoumarin" or "dicoumarol," in nontoxic doses reduces *in vivo* the coagulability of the blood of rodents, dogs, monkeys, and man. Except in rats, which are especially susceptible to this agent, there is a latency of at least twenty-four hours before either the coagulation time or the prothrombin time lengthens; the degree of prolongation and the recovery interval are related roughly to the dose (83, 84). In these more or less exploratory studies, two peculiarities deserve mention: (a) The very high levels to which the coagulation and prothrombin times rise without hemorrhage necessarily developing (83, 85); (b) the frequent lack of parallel changes of prothrombin and coagulation times (85, 86). It is likely that the prolonged coagulation time is dependent mainly on the defect of the first stage, but the coagulation defect may not be restricted to prothrombin deficiency (83). Other components of the blood do not appear to be affected unless hemorrhage develops (86, 87). Hemorrhage and dilatation of the finer vacular radicles are constant findings in death occurring by chronic intoxication; liver damage is frequent but not constant (86, 87, 88).

The use of dicoumarin in thrombosis, experimental and human, appears to be feasible (86, 87, 89, 90). The coagulation defect may be temporarily abolished by transfusion of fresh blood (86, 91). Vitamin K preparations in high dosage are antagonistic to the effect of dicoumarin (84, 92).

Sedimentation.—To the various factors which influence the sedimentation rate (93, 94, 95, 96), the purely technical one of anticoagulant and type of tube is added (97). A study of the electrophoretic pattern of the plasma relative to the sedimentation discloses a clear delimitation of normal from pathologic plasmas when the α-globulin/albumin ratio is plotted against the sedimentation rate (98). By application of the method of partial correlation, it is found that an increase of sedimentation rate is better correlated

with a low albumin level than with the decrease of hematocrit or rise of globulin or fibrinogen (99). This method of analyzing such data should be used more extensively.

Cells

Erythrocytes.—The chemical composition of the erythrocyte stroma is altered in certain pathologic states; the immature cell also varies from the normal adult cell (100, 101). Cabot ring bodies are considered to result from separation of lipoprotein layers of the cell envelope under hemolytic conditions and not to be preformed nuclear fragments (102). Following the injection of acetylphenylhydrazine, small refractile bodies—Heinz granules—appear in adult erythrocytes, not in reticulocytes. Cells containing them are less resistant to hypotonic saline (103). The acute intravascular hemolysis resulting from the drug is succeeded by an intense reticulocyte crisis composed of cells having volumes as large as 180 cu. μ; the cells probably lose the reticulation in three to five days. Heinz granules are detectible in the cells of patients treated with sulfa-drugs and are considered indirect evidence of methemoglobin formation (104). Their presence is coincident with the development of anemia (105).

The name "target cell" is given to erythrocytes which are bowl-shaped *in vivo*, and when stained resemble a bull's eye target (106). The shape of the target cell makes it more resistant to hypotonic salt solution and hence blood containing them requires a lower concentration for complete hemolysis; in this respect the cell can be regarded as the antithesis of the spherocyte. They are found in several types of anemias and systemic diseases as well as during the response to blood loss and in normal blood in low percentage. Target cells are conspicuous after splenectomy in man and animals (107) and following restriction of blood flow in the splenic vein, not the artery, in dogs (108). Other effects of splenectomy on the blood picture of man are described (107).

Human erythrocytes in hypotonic solutions behave as perfect osmometers (109). After hemolysis the ghost cell resumes its original form and contains about twice the hemoglobin expected at equilibrium; these findings imply an internal structure (110). No explanation is offered for the increase in size of the erythrocytes after hemorrhage (111).

Three reports agree that there are differences in the values for erythrocyte, hemoglobin, or both between peripheral and venous blood (112, 113, 114); the dissimilarity possibly is related to the flow of "lymph" from bleeding-time wounds (115) and the plasma sheaths of the blood vessels (8). By means of a dye dilution technique, the true volume per cent of cells is estimated to be about 4.5 per cent less than the hematocrit value because of incomplete packing (116). Measurement of erythrocyte number and hematocrit by opacity is unreliable (117).

Hemoglobin and iron.—The personal and observational error of hemoglobin estimations by the colorimetric techniques used clinically and in some research is such that changes of less than 3 gms. are dubious. However, photoelectric colorimeters once accurately calibrated are reliable and consistent (118). Calibration by oxygen or carbon monoxide capacity methods would not include the inactive fraction of the total hemoglobin (119).

Factors, except sex, which influence the rate of hemoglobin regeneration in healthy blood donors are not predictable. The administration of iron increases hemoglobin production for the first few regeneration periods although, when iron is not given, repeated donations do not influence the rate of regeneration (120). Studies using radioactive iron demonstrate that the iron liberated upon intravascular hemolysis is rebuilt into hemoglobin in preference to stored iron; thus the stored iron is reserve iron (121, 122). The porphyrin portion of hemoglobin is not reused (123); a new equivalence, 34.9 mg. bilirubin equal 1 gm. hemoglobin, is used in the calculations. In the anemic and hypoproteinemic dog, hemoglobin production seems to have precedence over plasma protein production (124). Actively growing tumors appear to divert materials needed for hemoglobin production (125).

Although the excretion of hemoglobin is an exception to the usual statements of glomerular permeability, it is not an exception to the normal mechanisms by which threshold substances are excreted (126). By the use of radioactive hemoglobin it is found that the tubule cells resorb hemoglobin and store decomposition products of it (127).

Following intravascular hemolysis, the intravenous injection of hemoglobin, and the incubation of sterile blood, there appears a pigment termed methemalbumin. It is regarded as a combination of serum albumin and hematin (Fe^{+++}) derived from methemo-

globin. Peculiarly, only human and simian plasma contains the specific albumin fraction giving the characteristic spectrum although ultracentrifuge studies indicate that hematin combines with the plasma protein of other species (128). The presence of methemalbumin is regarded as evidence of intravascular hemolysis (129). Intravenous injection of purified hematin, which has been identified as the pigment of malaria parasites (130), may result in either acute or chronic death; the pathologic changes in chronic animals, thromboses and kidney damage, resemble those of both malaria and transfusion accident (131, 132). Possibly it is significant that hemin and hemoglobin are destroyed during the *in vitro* oxidation of linoleic or linolenic acids while acting as catalysts (133). Several references are listed which indicate that the pH-precipitation theory of transfusion anuria is inadequate (134 to 137).

The presence of two types of hemoglobin in adult blood has been noted in several mammalian and nonmammalian species by the alkali-denaturation technique (138). The retardation of denaturation is ascribed to an inhibitory effect of the reaction products and carbon dioxide absorption (139). Solubility studies also point to two forms of hemoglobin in animals (140). By the precipitin technique both fetal and adult hemoglobin are present in cord blood, but only the adult type in adult blood (141). A carefully controlled study of the position of the oxygen saturation curve of fetal human blood relative to the maternal blood demonstrates that the former lies to the left of the latter at body pH. The curve is similar for premature and newborn infants; one month after birth the curve merges with the adult curve (142). Essentially the same relations and interpretations are found in the fetal and newborn calf (143, 144, 145). This property of fetal hemoglobin aids oxygen transfer under the conditions of pH and pO_2 *in utero*. A theoretical interpretation of the Bohr and Haldane effects in tautog blood reinforces the view that hemoglobin possesses separate oxygen-combining components; pH was not controlled (146).

Four references (147, 148, 149, 150) may be added to the recent comprehensive discussion of the physical characteristics of hemoglobin and related pigments (151). Other résumés (151, 152) make it unnecessary to mention studies of porphyrin metabolism other than to stress this method of attack on hemoglobin metabolism;

at present the studies are exploratory and semiquantitative (153, 154, 155).

Hemolysis.—A rigorous analysis of hemolysis produced by suboptimal amounts of saponin in the presence of accelerators together with other considerations favors the concept of a hemolysin first attacking scattered key points on the erythrocyte membrane rather than the preliminary formation of a monolayer (156). Such a concept does not conflict with conditions of excess lysin or spreading effect of suboptimal lysin concentration.

Oxidant compounds incubated with erythrocytes lead to intracellular formation of methemoglobin with spherocytosis and increased fragility, and injections of such compounds closely reproduce the features of the hemolytic crises in sulfanilamide therapy (157). The erythrocytes of some persons may contain as much as 40 per cent methemoglobin without systemic evidence of hemolysis, whereas in other persons hemolysis occurs more readily (158). By the use of radioactive iron, the newly delivered erythrocytes can be shown to be less resistant to hypotonic salt solution, but after about four days the difference is absent (159). The erythrocytes of animals exposed to low pO_2 or to high pCO_2 gas mixtures are more fragile to hypotonic salt solutions (160). Exposure of the erythrocytes to radiant energy of the visible spectrum accelerates hemolysis (161).

The recognition that the survival time of the erthrocytes in the recipient's circulation does not correspond with *in vitro* fragility by one or another test (162, 163) indicates that the best diluent for blood storage may yet be found; the older work on the merits of various diluents thus becomes of academic interest.

Reviews of the status of hemolytic anemias may be found elsewhere (7, 164, 165).

A series of studies on the protection by plasma against hemolytic agents typified by saponin, bile salts, fat acids, and cell extractives stresses the importance of cholesterol as an antihemolytic substance. The fact that drying the plasma causes it to lose some 25 per cent of its antihemolytic activity suggests that the physicochemical relations of its components are involved (166). By fractionation of plasma it is estimated that cholesterol and globulin account for about 40 and 15 per cent, respectively, of the total antihemolytic activity. Although lecithin alone (purity questionable) or combined with cholesterol is hemolytic in artificial systems.

the combination is more strongly antihemolytic than cholesterol alone when saponin or fat acids are present (167). The antagonism of cholesterol to saponin hemolysis is confirmed by the findings that the antihemolytic potency of the plasma of various animals is correlated with the free cholesterol content (168), that the antihemolytic activity varies with changes of cholesterol concentration induced in various ways (169) and with the spontaneous variations in human disease (170). Such *in vitro* estimations of antihemolysis must not be translated too literally to *in vivo* hemolysis (171).

Leukocytes.—A leukocytosis-promoting factor, which is nondialyzable, thermolabile, and associated with the globulin fraction, is extractable from the cell-free inflammatory exudates of animals and man; it is also found in the plasma of animals forming an inflammatory exudate. The leukocytic response by rabbits and dogs to intravenous injection of this substance is distinct from that of several materials which also produce leukocytosis, including nucleic acids and "leukotaxine." Since the leukocytosis is accompanied by an outpouring of young forms, the response is the result of bone marrow stimulation (172, 173, 174, 175). In line with these experiments are the reports that the intravenous injection of sterile saline-induced peritoneal exudate of rabbits is followed by a leukocytosis with left shift (176, 177). The latter experiments are especially well controlled by studies of the leukocyte counts of normal rabbits. Each animal appears to have its own fairly constant leukocyte level and weighted mean index of maturity of amphophils (178, 179).

The names "propolycyte" and "polycyte" are given to normal size neutrophils having six and more than six segments, respectively. The smaller size of these cells distinguishes them from Cooke's macropolycytes. These cells have been found in the blood of about 10 per cent of persons toward the end of a period of left shift. The suggestion is made that the development of nucleus and cytoplasm may be influenced by separate factors, an hypothesis which would explain the divergent views on the length of life cycle of the neutrophil (180). Agglutination of leukocytes and bone marrow suspensions by bacterial toxins is correlated with loss of ability to reduce methylene blue. The results indicate that leukoagglutinin and leukocidin are but two stages of action of the same toxic substance (181).

Reference is made to reviews of the basophil (182) and the acidophil (183). The participation of the leukocytes in anaphylaxis is considered in an extensive review (184).

A significant paper dealing with the error in making differential counts should be examined by those engaged in this field (185).

An analysis of the preoperative leukocyte count relative to the pathology of the appendix demonstrates that it is more logical to regard the white count as a measure of the patient's resistance rather than the state of the appendix (186). A syndrome designated "primary splenic neutropoenia" is described which is analogous to hemolytic jaundice and thrombocytopenia; thus the picture of hyperphagocytosis by the splenic cells is rounded out (187).

Calculations made on the basis of the number of lymphocytes entering the circulation by the thoracic duct imply that the population of lymphocytes in the blood experiences a daily turnover of 0.5 to 5.0; the true figure probably is higher since there are other points of discharge (188). Lymphocytes can live longer in the tissues than the preceding data suggest as evidenced by observation in ear-window preparations (189). In dogs, removal of the intestines has no immediate effect on the number of circulating lymphocytes (190); thus no information is obtained as to whether the gut is the source or exit portal for lymphocytes. Radioactive phosphorus progressing through the gastrointestinal tract reduces the number of circulating lymphocytes (191).

Platelets.—A study (192) of the "stickiness" of platelets under controlled conditions ***in vitro*** demonstrates that the higher the concentration of several anticoagulants, the slower is the disappearance of the platelets from the blood. The platelet count drops sharply during the first ten to twenty minutes in logarithmic fashion, suggesting, perhaps, a distribution of the "stickiness" property in the platelet population. Semiquantitative data are offered that the platelets adhering to the glass section of the chamber account for the decrease, but it is impossible to estimate the degree of disintegration subsequent to agglutination. Anticoagulants, moreover, affect the appearance of platelets in dark field observation (193). In the dark field, fibrin needles arise only from platelet clusters disintegrating at foreign surfaces, never from the free-floating clusters (194). Another report does not concede that platelets undergo lysis at foreign surfaces (195).

The blood platelets decrease abruptly in the blood of animals during the antigen-antibody response of anaphylactic shock (196). The sharp decrease is regarded as one of the factors of the immediate increased blood coagulability as well as contributing histamine (197). Extensive experience (198) with the platelet number and coagulability of platelet-containing and platelet-free plasma has led to the opinion that the activity or disintegration ability of the platelets is of more consequence than the absolute number. Where the platelets are "normal," numbers in excess of 30,000 per c.mm. make little difference in coagulation time, and this is true during the postoperative rise of platelets (199); it is only when the platelets are below 30,000 per c.mm. that the coagulation times lengthen (198). Another report places the level at about 50,000 per c.mm. (200). In the newborn the platelets remain within normal limits although the coagulation time of both platelet-containing an platelet-free plasma lengthen on the third day (201). No explanation is offered for the not greatly prolonged coagulation time of platelet-free plasma.

The presence of a thrombocytopenic material in the spleen of patients with thrombocytopenic purpura still is not proved (202, 203, 204, 205). An hereditary hemophilic state in swine is reported (206,207) in which the defect, as in man, involves the availability of plasma thromboplastin. A résumé of the clinical aspects of hemophilia, thrombocytopenia, and the purpuras may be found in comprehensive reviews (7, 208).

Bone marrow.—Investigations of the energy metabolism of marrow cells demonstrate that the type of respiration found depends on the erythroid/myeloid ratio. The erythroid elements have higher rates of respiration and lower rates of anaerobic glycolysis than myeloid cells. Under conditions of low pO_2, glycolysis increases reciprocally with the decrease of respiration, but the critical pO_2 is very low, approximately 4 mm. Hg (209, 210).

A novel technique of bone marrow culture is described, the feature of which is the continuous passage of air over a coagulum implant. The air is filtered but not saturated with water vapor (211). However, good results are reported.

Several studies of hematopoiesis have appeared (212 to 217), but due to diverse terminology and views they can not be reported briefly. Precocious hemoglobinization of erythroblasts is noted under conditions of demand for erythrocytes and is not character-

istic of the megaloblast (212). The use of a vital stain on the marrow cells of frogs and birds makes evident the appearance and disappearance of reticulation coincident with "necrobiotic" changes of the nucleus. If the reticulation arises from the nucleus as illustrated, the status of polychromasia must be reconsidered (218). It is claimed that vital staining of the mouse embryo blood cells permits clear differentiation of the early "megaloblastic" from the succeeding normoblastic lineage; the cells of pernicious anemia bone marrow and the early stage are considered similar if not identical (219). In human normoblastic maturation the nucleus disappears before the reticulation disappears, whereas in megaloblastic ripening the reverse happens. Both absorption and extrusion of the nucleus occur (220).

Detailed data on cellular changes of the blood of the human fetus (221) and newborn (222) and the bone marrow of the newborn (223) must be examined in relation to the discussions presented in those reports, as is true of an extensive study of the bone marrow in anemia (224).

A classification of the myelogenic anemias refractory to any type of therapy is proposed. A separation into four groups is based on the qualitative and quantitative state of the bone marrow without distinction as to disease or agent responsible (225). This is the first comprehensive study in a relatively new field; it should serve as the point of departure for future work. A discussion of this subject from the clinical diagnostic view fits nicely with the preceding citation (226).

Rh factor.—The recognition of the etiologic relation of the Rh factor to erythroblastosis fetalis probably represents the signal advancement of the year in hematology. The Rh factor, a type factor like M and N, is present on the erythrocytes of some 85 per cent of white people and is transmitted as an autosomal dominant (227). Compelling statistical evidence has established the isoimmunization theory (228): the cells of the Rh-positive fetus serve as the antigen to which an Rh-negative mother forms the antibodies believed responsible for the triadic syndrome recognized clinically as erythroblastosis fetalis. This immunologic relation may be responsible for other complications of pregnancy and other antigens, at present unidentified, similarly are implicated (228, 229). Further, the Rh factor is responsible for the majority of true intragroup transfusion reactions (228, 230). The

clinical implications are reviewed elsewhere (231). The theory as applied is not without unanswered questions (232).

Pernicious anemia.—The variance between the site of intrinsic factor formation by the "pyloric gland organ" in swine and the pathologic findings in pernicious anemia proves to be a species difference (233). Only the extrapyloric regions of the human stomach, which contain the gastric crypts, supply the intrinsic factor. Atrophy of the mucosa of the body of the stomach is a constant feature in gastroscopic examination in pernicious anemia, and remission is not accompanied by restoration of the mucosa. Achlorhydria and atrophy of the gastric mucosa are not pathognomonic of pernicious anemia (234).

A proposed bioassay method for the antipernicious anemia factor of liver extract (235) depends on the centrifugal migration of cells from a slice of bone marrow embedded in various dilutions of liver extract in a plasma coagulum. While there is no evidence that the response is specific for the antipernicious anemia factor, the relation between the pharmacopoeial unitage (which itself is crude) and the "optimal migration dilution" is remarkably close. Ventriculin added to the diet of pregnant rats is reported to result in a significant decrease in diameter of the cytoplasm, of the nuclei, or of both in fixed preparations of the prehepatic erythroblasts of the fetuses (236). It is not clear why the data of some litters are significant while the data of similarly treated litters are not unless the time of sampling, "11 days," is critical. The relative degrees of fetal maturity are not stated. Control and inactive extracts are described as ineffective (237). Another laboratory is unable to confirm the effect of ventriculin on nuclear size, and, further, liver extract injected into the pregnant females is also without effect (238). Concentrated human gastric juice and liver extract injected into immature pouch young of the opossum is reported to accelerate the transition from the fetal toward the adult status of erythrocyte number and size (239). The syllogistic control method renders the data inconclusive, particularly since the effect of liver extract is not confirmed (238). The erythropoietic response of the chick embryo to injected liver extract is a nonspecific effect (240).

A critical analysis of the present ill-defined method of assay of antipernicious anemia activity in liver extracts points out that the reticulocyte response is not specific and is not well correlated

with the potentialities of the bone marrow (241). The proposed criteria of assay, the serum iron and cellular changes in the bone marrow, deserve examination for potential use. The effect of liver extract on the megaloblastic bone marrow is evident as soon as three hours after injection and the reappearance of the normoblastic marrow proceeds at an equally rapid pace, but the frequency of mitoses is not increased (216). The quantitative data presented (216) may be related to either interpretation of the maturation defect.

To a review (242) of the knowledge of the composition of the antipernicious anemia principle, there may be added the data on a fraction (243) which is active in total doses of less than one milligram. Although of the isolation schema involves several innovations, the fraction possesses notable similarity to the fractions prepared otherwise. Attempts (244, 245) to produce the pernicious anemia syndrome by various resections of the gastrointestinal tract have not been successful; an outstanding finding is the tendency of the animals to develop achylia gastrica and a pellagrous condition with central nervous system involvement.

The relation of the vitamin B complex to the integrity of the central nervous system, gastrointestinal tract, and bone marrow (246 to 250) suggests participation of these substances in the pernicious anemia syndrome and clinical evidence sustains this (247, 251, 252, 253). Absorption of two B-components, pantothenic acid and riboflavin, is not abnormal in patients with pernicious anemia during relapse (254, 255).

It is proposed (256) that the residual secretion of intrinsic factor accounts for the variability of data in attempts to estimate the storage capacity of the liver (257) for injected antipernicious anemia preparations. Probably both the residual secretion and the storage of a part of the injected extract explain the ability of patients to remain in remission when given concentrated extracts at intervals of four weeks (258, 259). The status of the patient is not improved by administering more than maintenance doses (259).

Polycythemia.—Cobalt is generally regarded as a polycythemic agent, but the normal erythropoietic response of pups and phlebotomized dogs on a milk and salt-mixture diet is inhibited by cobalt (260). This finding is corroborated in anemic dogs on a salmon-bread diet (261). Nevertheless, small amounts of cobalt appear to supplement the iron and copper in erythropoiesis (262,

263). Polycythemia results when certain vasoconstrictor drugs are administered to normal and splenectomized animals, and ephedrine enhances the polycythemia induced in rabbits by cobalt (264, 265). When vasodilator drugs are given during the polycythemia, the erythrocyte level very abruptly drops; on discontinuing the drug, the count rises sharply (264). The administration of nicotinic acid amide prevents the development of polycythemia in rabbits (266). The recovery from low pO_2 polycythemia depends on decreased marrow activity rather than increased destruction (267).

Endocrines.—The relation of the endocrines to the blood picture is the subject of a number of papers which must be read in the original, for it appears that species and endocrine status of the animal and dose of the preparations employed influence the data. One fact is clear: administration of large doses of estrogens and physiologically equivalent amounts of stilbesterol result in early leukocytosis followed by the blood picture of panmyelohypoplasia (268 to 274). Small doses of estrogens may raise the erythrocyte levels (268). Monkeys have high resistance to the estrogen depression (274, 275) and other animals, which are reactive, develop a certain habituation (268, 271). Androgen administration results in increased erythrocyte counts in rats (269) and man (276), but not in dogs (273). That the marrow hypoplasia by estrogen and hyperplasia by androgen in rats (269) is not purely a drug effect is shown by typical response of normal and hypophysectomized, but not castrated, rats to a gonadotrophic extract (270). However, the use of comparatively high doses of hormone preparations in these studies warrants deferred evaluation. Additional critical data are required before conceding a specific regulatory influence by the gonad hormones on hematopoiesis and the circulating blood. The same applies to studies on the effects of other endocrines (270, 277).

Leukemia.— The studies preliminary to optimal use of radioactive phosphorus in blood diseases revolve about a better knowledge of phosphorus metabolism. In general, it appears that tissues with high phosphorus content or high rate of phosphorus metabolism retain radioactive phosphorus in amounts sufficient to be of therapeutic value (278 to 284). Thus, cells of bone marrow, lymph node, and certain neoplasms retain or are exposed to effective "differential absorption ratios" (278) of radioactive

phosphorus. While there can be no doubt that the energy of the β-particles produces the destructive changes, it is interesting to speculate on the consequences to a molecule which suddenly finds itself with a protean atom, i.e. ${}_{15}P^{32} \rightarrow {}_{16}S^{32}$.

Another method of differential tissue destruction is described (285) which depends on the capture of slow neutrons by lithium and other elements, the disintegration products of which produce tissue damage.

Additional evidence is presented (286) that leukemia in mice is inherited, probably on a multiple factor basis with undetermined environmental influences. The susceptibility of foster mothers to leukemia is reported to influence the susceptibility of the nursed young (287, 288). A statistical correlation of the pretransplant blood cell formula of the rabbit with the frequency of takes of the Brown-Pierce tumor shows that the hemoglobin and leukocyte levels are critical; if either is not modal, the animals are more susceptible (289). Other factors are related to the frequency of metastases (290). The opinion is expressed that leukemia in man and animals, especially in mice, is the same disease (291), but the cells of induced leukemias differ genetically from the cells of spontaneous leukemias as judged by transplant attempts (292). An important contribution to the understanding and recognition of the lymphatic leukemias is the concept of a type probably metabolic in origin (293).

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THE LYMPHATIC SYSTEM

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The present paper reviews most of the work upon the structure and the activity of the lymphatic system that has appeared in the interval between the summary of the subject by Warren (1) in the *Annual Review of Physiology* and September 1, 1942. All papers published during this period have not been discussed nor have those been included which have to do mainly with the surgical pathology of the lymphatic system, or with the lymphadenopathies, or with the metastasis of tumors by way of the lymphatics. Under the stress of present times some important works may have been overlooked. On the other hand, some papers have been summarized not because they contain important new data but for the reason that they present unaccepted opinions or revive abandoned views, and so serve to give the reader a total picture not only of current work but of current thought about the lymphatic system.

Recent Advances in Anatomical Knowledge

Since physiological understanding of lymphatic function often waits upon anatomical knowledge, it may be well first to review a few anatomical studies which have special interest to the physiologist.

Wislocki & Dempsey (2) have remarked upon the functional significance of the lymphatics of the female reproductive tract in rhesus monkeys. Injection studies have shown that the rich lymphatic plexuses of the nongravid uterus become hypertrophied and dilated during pregnancy. The authors suggest that, by drainage of the protein-containing tissue fluids, the lymphatics of the reproductive tract remove the physiological edema appearing in these tissues during the active phase of the estrus cycle.

Parker (3) has gone far toward the answer to the question: Do the lymph channels of the human bladder and kidney connect with each other by way of the ureteral lymphatics so that the latter serve as pathways for ascending infections of the urinary tract? By a series of injection studies on the stillborn she found that the lymphatic capillaries of the ureters give rise to draining channels

that eventually leave the ureters to pass to regional nodes, a state of affairs not previously reported. There is no indication of a complete network of lymphatic channels throughout the walls of the ureters. However, the posterior abdominal lymph nodes act as regional nodes for both the ureters and other organs of the urogenital tract. The possible practical significance of lymph drainage from the ureters, kidneys, and bladder to regional nodes has not yet been worked out experimentally.

Webb & Simer (4), by intraperitoneal injections of a certain brand (Hollborn) of 1 per cent trypan blue solution into rats, guinea pigs, rabbits, and dogs, rendered the omental lymphatics visible and traced them into areas of milk spots to show their relations to the latter. On the other hand, Mixter (5) has demonstrated the presence of "milky spots" in the pleura of several mammals and man. Reconstructions of the vascular relations in these macrophagal foci, as he terms them because of the predominance of macrophages, and other studies have failed to show the presence of lymphatics. The author contrasts these foci which seem to be drained wholly by the blood stream with lymphoid tissue structures elsewhere in the body which are drained chiefly by lymphatics.

Recently interest in the lymphatic wall has been revived. Clark & Clark (6), in a study of the growth of lymphatic and blood capillaries within the Clark chamber in the rabbit's ear, have reported that lymphatic endothelium like that of the blood vessels is composed of an exoplasm and an endoplasm, the former existing as a continuous, homogeneous layer, the latter containing the endothelial nuclei and surrounding cytoplasm. Further (7), the lymphatics in the chambers did not seem to participate in the removal of fat from the tissues or in its transport to them from the blood, for no relationship was observed between the presence or absence of lymphatics and the appearance or disappearance of fat in the tissues.

Pfuhl (8, 9, 10), studying lymphatic capillaries of guinea pig skin after injection of dye and fixation, found even the smallest lymphatics equipped with valves. He found no pericytes, no stigmata or stomata, and no perilymphatic capillary spaces. He considers the latter when they appear in sections to be edema fluid artifacts, pathological or post-mortem changes, or, if they appear close to blood vessels, as collapsed lymphatics. He found the lym-

phatic capillary endothelium always backed by a fine basal membrane, which stems from the endothelium and from which the valves come, covered on both sides with endothelium.

Pfuhl & Wiegand (9), by intraperitoneal injections of 1 per cent trypan blue, have rendered the omental lymphatics visible in the guinea pig, in which, as is well known, the abdominal lymphatics are contractile. They describe collections of smooth muscle cells about the bulbous portions of even the smallest lymphatics and, calling them micro-lymph-hearts, attribute great importance to them as furnishing motive power for lymph flow. They believe that omental lymphatics do not take up cells or particulate matter but that their special function is the taking up of soluble substances, especially proteins, and they describe the presence of protein flocculi in living and also stained lymphatic channels.

Factors Influencing the Composition and Flow of Lymph

Cholinesterase.—There is in lymph an enzyme that behaves like cholinesterase. Friend & Krayer (11) have devised an ingenious method to measure its activity in body fluids and tissue extracts. In the dog its activity is greater in thoracic duct lymph than in cervical lymph but still greater in serum than in either. Its activity is inhibited by prostigmine methlysulfate.

Lymph as an ultrafiltrate from blood.—To test whether or not lymph and certain other body fluids are ultrafiltrates from the blood, Robertson & Williams (12) injected hypertonic solutions of glucose, urea, and creatinine intravenously into cats and found these freely diffusible substances in approximately equal concentrations in the lymph and plasma after their concentration had been raised in the blood. This did not follow for the aqueous humor.

Knowledge of the constituents of body fluids other than lymph is of importance in gaining an understanding of lymph formation. Maurer, Warren & Drinker (13), collecting blood-free samples of pericardial fluid from various laboratory animals and from human autopsies, have shown from studies of the protein content, the albumin-globulin ratio, and the chlorides that the pericardial fluid is probably an ultrafiltrate of blood serum. By studying the passage of horse serum protein or acacia from the blood to the pericardial and peritoneal fluids of the dog, it was shown that the protein of both fluids is probably derived by diffusion from the protein of the blood. The exchange of substances from blood to lymph has

been further clarified, as the summary of the next few papers will show.

Heat and cold and lymph flow.—Earlier work on the formation and flow of lymph not recent enough to be reviewed here has shown that circulatory changes following applications of heat or cold affect the flow of lymph. Recently McCarrell (14), employing her "nodding dog" preparation described in an earlier review (1, 15), has measured cervical lymph flow in dogs following nasopharyngeal irrigation with isotonic saline solution at various temperatures. The temperatures that produced changes in lymph flow were those which are known to effect blood vascular changes. Irrigation at 5°C. yielded an increased lymph flow which was temporary or prolonged depending upon whether or not blood capillary damage had been effected. At temperatures between 5° and 45°C. there was no marked effect on lymph flow. At 45°C. lymph flow increased but only as long as the heat was applied. Lymph protein did not increase, indicating that the permeability of the blood capillaries had not been affected. Irrigation fluid temperatures higher than 50°C. greatly increased the flow of lymph and its protein content. The effect was irreversible, evidently resulting from capillary damage. It is of interest to note in this connection that McMaster & Hudack (16) found the lymphatic capillary permeability in the white mouse's ear increased by a temperature of 43.5°C., approximately that at which McCarrell found increased lymph flow beginning. They also found (17) that temperatures of 53° to 55°C., applied to the ear for less than a minute, brought about severe changes in lymphatic capillary permeability.

McCarrell describes (18) the effect of changes in environmental temperature upon cervical lymph flow of dogs and cats. In a hot environment, when the body temperatures remained between 38.3°C. and 41.1°C., cervical lymph flow increased and the percentage of lymph protein decreased, an accelerated rate of capillary filtration presumably following arteriolar and capillary dilatation and increased capillary pressure. When the body temperture was restored to normal levels lymph flow returned to normal but when the body temperature was allowed to rise to levels between 41.9°C. and 43.5°C., the flow of cervical lymph suddenly increased three to eighteen times, the arterial blood pressure fell and circulatory collapse became imminent. The excessive lymph flow appearing prior to collapse seemed to follow a temporary in-

crease in venous pressure and an increased capillary permeability brought about by stasis and anoxemia. The volume of lymph flow was so great that the increase in lymph protein percentage usually accompanying capillary damage was absent. In a cold environment no striking changes appeared in cervical lymph flow, even when the body temperature was reduced to 25°C. or 26°C.

Factors affecting the flow of cardiac lymph.—Drinker, Warren, Mauer & McCarrell (19), collecting lymph from the entire heart of the dog, found the flow constant during constant circulatory conditions, but variable from animal to animal. Increased work of the heart following administrations of epinephrine or ephedrine increased the lymph flow, but sodium nitrite, lowering the blood pressure, decreased the flow. The cardiac blood capillaries were found permeable to protein, for both horse serum and acacia appeared in the lymph following their injection into the blood stream. Plasmapheresis, reducing the blood proteins greatly, increased lymph flow. This work together with that already cited (15) shows from comparisons of the protein content, the albumin-globulin ratios, the colloid osmotic pressures, and the chloride concentration of cardiac lymph and pericardial fluid, that both are clearly filtrates from the blood.

The effects of oxygen lack.—Maurer (20) has studied in the dog changes in the flow and the composition of cervical and cardiac lymph brought about by decrease in blood oxygen, increase in blood carbon dioxide, and by carbon monoxide anoxemia. Oxygen lack began to increase the flow of lymph when the blood oxygen saturation fell to 75 per cent. This is the saturation at which other physiological changes begin to appear and is like that found at an altitude of 17,000 feet. Lymph flow became greatest when the blood became about half saturated, as it is at an altitude of 20,000 feet. As long as lymph flow remained constant, lymph protein remained constant. When lymph flow increased the output of protein varied with the rate of flow, although the percentage in lymph protein fell. The ratio of lymph albumin and globulin remained constant. Acacia injected intravenously appeared in the lymph and increased sharply under conditions of low oxygen tension at the same time that the flow of lymph increased. As the latter took place while the blood pressure fell there must have been an increased escape of fluid from the blood. High blood carbon dioxide tensions had similar effects. Maurer believes that the low oxy-

gen or high carbon dioxide content of the blood increased capillary permeability. Further evidence of this was found in an increase in the concentration of the plasma proteins.

Maurer found further (21) that the cervical lymph flow of dogs and a cat breathing air containing 0.5 per cent carbon monoxide increased 2.42 times, whereas exposure to low oxygen increased it 2.7 times (20). During both oxygen deprivation (20) and carbon monoxide inhalation (21), lymph flow increased when the blood oxygen saturation fell to approximately the same level. In both types of anoxemia, when lymph flow increased, the percentage of lymph protein decreased but, although the blood pressure fell, the total protein output in the lymph increased and the serum protein percentage decreased. Maurer suggests that the effects of carbon monoxide inhalation are produced by oxygen deprivation rather than by the carbon monoxide itself.

Factors affecting the flow of lung lymph.—Warren & Drinker (22) have demonstrated crossed lymphatic drainage from the lungs in dogs and have cannulated a lymphatic which carries lymph from all parts of the lungs except a portion of the upper right lobe. Lung lymph appeared to be much like lymph from the heart, having an average protein concentration of 3.66 per cent and an albumin-globulin ratio like that of blood and lymph from other organs. The flow of lung lymph collected during artificial respiration decreased when the ventilation was increased. A continuous insufflation with oxygen, which held the lungs quietly distended, also decreased lymph flow. The authors speculate upon what might happen if artificial respiration, like natural respiration, could be effected by the exertion of negative pressure in the chest. Under these circumstances lymph flow might be increased by a sort of massage of the lymphatics. Oxygen lack led to a sharp increase in the flow of lung lymph, the finding suggesting to the authors an increase in blood capillary permeability resulting from this lack. Constriction of the pulmonary veins for ten minutes, during which serious oxygen lack in the blood was avoided by ventilation with pure oxygen, produced a large increase in the flow of lung lymph, which began after the inrush of blood to the lungs had occurred following the release of obstruction.

Gall bladder and liver lymph.—While studying the movement of lymph in the upper abdomen, McCarrell, Thayer & Drinker (23) cannulated simultaneously lymphatics of the gall bladder and

liver of the cat. They report an extraordinary similarity in the protein content of serum, gall bladder, and liver lymph, which were found on the average to be 5.74, 5.61, and 5.88 per cent, respectively. The intimate connection between liver and gall bladder lymphatics has been demonstrated by Sappey (24), and by Baum (25) as noted by the authors, and also by Sudler (26) and by Bartlett, Crile & Graham (27, 28).

The exchange of dye from blood to lymph.—The exchange from blood to lymph has been studied in other ways. Ferrebee, Leigh & Berliner (29) found the blue dye T-1824 in cervical and thoracic lymph within the hour following intravenous injections of only 1 cc. of a 1 per cent solution into dogs. They estimate that 75 per cent of the injected dye and 15 per cent of the plasma protein passed through the thoracic duct in two hours. They state that this rapid loss of dye into the lymph disturbs the accuracy of blood volume determination by the blue dye method, but can be corrected for under normal circumstances. Under abnormal and varying conditions, as for example in shock, the application of the correction may be difficult.

The effect of the pulse on the flow of lymph.—In various lymphatics of the dog, Cressman & Blalock (30) have recorded pulsations synchronous with the arterial pulse. The lymphatic pulsations were present even in undistended lymphatics. Noting the fact, they discuss the possible effect of the pulse upon the flow of lymph and conclude that arterial pulsation is probably of importance in promoting lymph flow in the resting organism. They refer to earlier work by Beck (31), Sainsbury (32), and Clark & Clark (33), indicating that changes in arterial caliber can affect the movement of lymph, and cite also the experiments of Parsons & McMaster, in which, during pulsatile perfusions of blood in the rabbit's ear, there was more rapid lymph flow (34) and a greater spread of interstitially injected dye (35) than during nonpulsatile perfusions.

The Transport of Substances in Lymph

Cutaneous lymph movement in man and animals.—Intradermal injections of toxins and bacterins form the basis of many immunological tests, such as the Schick tests or skin tests for allergy. The role of cutaneous lymphatic drainage, not only in the local cutaneous response but in the initiation of antibody formation in lymph nodes nearest to the site of injection, has been recently reviewed by

the author (36). Abramson & Engel (37) have stressed the activity of the minute cutaneous lymphatics of man in the mechanism of the spread of histamine wheals, which form not so much by diffusion of histamine through the tissues as by the convection of it by the minute lymphatics. From the latter, at some distance from the original injection site, histamine escapes into the tissues to act upon the blood vessels. Some of it may be taken up again into the lymph and carried a little farther through these channels, to escape again and to act in the tissues at a still more distant site. Furthermore, Abramson & Gettner (38) have introduced histamine and epinephrine into human skin by electrophoresis, with current densities so low that no appreciable tissue injury occurred. In the skin of the forearm epinephrine caused blanching and vasoconstriction, and presumably a decrease in dermal tissue fluid pressure. Nevertheless, the substance was taken up by the intact lymphatics and drained up the arm in the same way that dyes are after they have been injected intradermally (39, 40, 41).

Griffith, Roberts, Rutherford & Corbit (42), studying cutaneous lymph flow in man by the method described by McMaster (40), found it either increased or normal in patients with high blood pressure. Increased lymph flow when present was accompanied by raised minute vessel pressure, and was considered the result of the high capillary pressure. In rats rendered hypertensive by administration of water and an antidiuretic dose of pitressin, cutaneous lymph flow seemed to be normal. The authors attributed the finding to the prevention of excess capillary pressure by the arteriolar constriction. In certain patients (43), in whom both the blood pressure and the blood volume were greater than normal, there was present either increased minute vessel pressure or increased cutaneous lymph flow or both.

The effects of roentgen irradiation upon cutaneous lymph flow.—Hodes & Griffith (44), after treating the hind legs of rats with x-rays, found no appreciable interference with lymph flow, as evidenced by injections of thorotrast. Ané & Burch (45) irradiated human skin with 220 to 450 *r* and found no effect upon cutaneous lymph flow, as tested by the dye injection method (40). In one patient receiving 1,187 *r* there was a decrease after a first degree skin reaction had occurred.

Lymphatics of the nasopharynx and intracranial nasal communications.—In work already reviewed by Warren (1), Drinker and his co-workers pointed out that dyes placed on the mucosa of

the nasopharynx appeared in the cervical lymphatics in the blood but did not appear in the cranium. Yoffey (46), extending these previous studies, has reported that, although color appeared in the cervical lymphatics of cats twenty to thirty minutes after placing a 5 per cent solution of the dye T-1824 in the nose, very little color appeared if the 5 per cent solution was made up in horse serum. When a 1 per cent solution in horse serum was used, under which conditions the dye is absorbed on the protein, no color appeared in the cervical lymph. Horse serum proteins placed in the nose did not appear in the lymph either.

Investigations of the relationship of lymphatics of the upper nasal cavity to the fluid of the subarachnoid space have received further investigation by Yoffey & Drinker (47). Injections of India ink, or of potassium ferrocyanide and ammonium citrate, into the subarachnoid space in dogs, cats, and a monkey passed downward along the olfactory nerves separating the sheaths from the fibers until, if pressure was sufficient, the fluids passed along the nerves to the mucous membrane. They found that the rich plexus of lymphatics of the nose, lying in close contact with the nasopharyngeal epithelium, could be injected from the cranial subarachnoid space. On the other hand, foreign substances did not pass readily in the opposite direction, for the neural sheath fits closely against the nerves and pressure only tended to press it more tightly against the nerve filaments. Drinker (48) notes further that in the dog with cannulated cervical lymphatics there was no evidence of lymphatic absorption of the dye T-1824 after it had been instilled into the frontal sinuses.

Movements of abdominal lymph.—Little & Robinson (49), in a careful study, have reinvestigated the role of the lymphatics in the transport of lipids absorbed from the intestine. In dogs with the left thoracic duct cannulated and the right duct blocked, cream of known lipid content was injected into the duodenum. The rate of lymphatic transport of total lipids, neutral fat, and phosphatides increased, but only 4 to 17 per cent of the lipids actually absorbed from the intestine was collected in the lymph. The authors' findings indicate that neutral fat and phosphatide entered the general circulation during lipid absorption. As there seemed to be no evidence that the absorbed lipids reached the portal blood directly, they suggest that they entered by way of lymphaticovenous communications.

Freeman, Loewy, Marchello & Johnson (50), in studying the

bile pigment output of dogs on high fat diets, have confirmed their earlier findings, already reviewed (1), that thoracic duct lymph becomes hemolytic after a fat meal.

Simer & Webb (51) have made the interesting observation that omental lymphatics in omental-jejunal adhesions take on the functions of lacteals in the transport of fat. Feedings of fat stained with sudan IV (not sudan III) to dogs, in which omental-jejunal adhesions had been produced by surgical means, led to physiological coloration of the chyle which followed its usual path from the central lacteals to the plexuses of lymphatics in the longitudinal muscle and in the serosa, and thence through the new formed lymphatic connections to omental lymph channels.

Hurst (52), writing on the pathogenesis of the sprue syndrome, has suggested obstruction of the lacteals by paralysis of the muscularis mucosae as the cause of the characteristic features of the condition. Together with Wright & Ryle (53) he has reported a case of sprue syndrome with severe steatorrhea resulting from obstruction of the lacteals by tuberculosis of the mesenteric lymph nodes. .

Lymphatics of the kidney.—Kaiserling & Soostmeyer (54), studying the effects of unilateral lymphatic obstruction of the rabbit's kidney, report the volume of the organ doubled in ten to twenty minutes and the secretion of urine, of low specific gravity, approximately trebled. The kidney's ability to concentrate sodium chloride or urea given by mouth decreased although, owing to the increased urinary output, the absolute amount of each in the urine increased.

Kaiserling (55) makes the unwarranted statement that he is the first to perform qualitative and quantitative studies on kidney lymph [for this error, see Schmidt & Hayman (56) who worked in 1929 and 1930], and further seems to believe that he is the first to tie off lymphatics from an organ to dilate them for subsequent study [see the work of Lee (57) done in 1923]. However, he has raised interesting questions which need to be investigated. He rightly contends that renal physiology has been studied with insufficient regard for the lymphatics. He believes the kidney to be so rich in lymphatics that distrubances of kidney lymph flow can lead to severe disturbances of kidney function, as shown by the development of interstitial edema and later nephrosis following lymphatic obstruction.

After obtaining renal lymphatic obstruction, Kaiserling injected, near the kidney hilus, either pig serum, in sensitized animals, or streptococci. In a few of many experiments lymphangitis resulted and in two instances extended to the smallest lymph capillaries of the kidney parenchyma. Microscopic sections of the experimental lymphangitis showed a great likeness to certain renal pathological changes, for example, chronic tuberculosis or ascending infection from pyelitis. Processes which appeared like cellular reactions in the interstitial tissue or like perivascular inflammation were in reality found to be the results of lymphangitis. Kaiserling insists that renal lymphatic capillaries are plentiful but cannot be distinguished in histological sections. They have, therefore, received little attention and have usually been mistaken for "tissue spaces" or "lymph spaces."

With Fischer (58) he has reported that injections of serum into the abdominal lymphatics of sensitized rabbits led to the production of an inflammatory edema which developed after two days into a diffuse leucocytic infiltration of all neighboring tissues. When the injections were made into the lymphatic capillaries of the gall bladder, all stages of cholecystitis followed; when afferent gall bladder or liver lymphatics were injected, changes are reported to have occurred in both abdomen and chest like those observed in the human pathology of pancreatitis, perihepatitis, and pericarditis, and even a sympatheticoganglionitis followed.

Lymphoid Tissue and Lymphocytes

Krumbhaar, writing on the changes occurring within the lymphatic system in the ageing organism (59), summarizes some of the more recent findings from a study of the spleens of three hundred persons who met death by violence. The findings indicate that the Malpighian follicles and lymphocytic tissue are low at birth, reach a peak before the tenth year, fall sharply in the second decade and until the fiftieth year, and then increase until the sixty-fifth year, to fall again subsequently. The spleen increases significantly in weight between the fifty-first and sixty-fifth years and thereafter falls. However, even in extreme old age, lymphocytic tissue may persist in considerable amounts, as is apparent from the spleens of persons ninety to ninety-five years of age. Krumbhaar suggests that we should reserve as a possibility the occurrence of a real increase of lymphoid tissue in late middle life and that this

may be associated with increased resistance to various infectious processes.

Hellman (60), in reviewing his recent work and that of others on lymphatic tissue in man, places the high point in the development of reaction centers between the tenth and twentieth years. He associates the reaction centers with the establishment of immunity and cites the work of Sjövall (61) to show that they have little to do with lymphocyte formation, and also that of Glimstedt (62) who found none in guinea pigs raised under sterile conditions.

Kindred (63), by an ingenious method, has calculated in two groups of albino rats, fifteen and twenty days old, respectively, the production of small lymphocytes in parotid lymph nodes, spleen, bone marrow, and thymus, and the needs of these organs for lymphocytes during growth. He concludes that the lymph nodes during this period do not produce excess lymphocytes to be filtered out in the bone marrow or spleen to serve as ancestors for blood cells. The thymus may be much more important in the early stages of the development of lymphoid organs than has been suspected, for, assuming that the mitotic cycle is one hour, the production of lymphocytes by the thymus is equivalent to 20 per cent of the small lymphocyte population of the blood. This figure compares with that found by Yoffey (64) for the number of lymphocytes entering the blood stream every hour from the thoracic duct of the dog. Kindred believes that these cells, needed by the growing lymph nodes and lymphoid tissue, do not become the ancestors of blood cells. The spleen and the bone marrow can meet their own needs for growth, at this period of life; only the cortices of the lymph nodes and the blood need small lymphocytes, and sufficient are formed by the thymus.

Jordan (65) writes that the appearance of lymph nodes taken at autopsy from a case of aplastic anemia suggested exhaustion in the effort to furnish small lymphocytes and that the nodular areas lacked germinal centers. The spleen's appearance indicated the end phase of a long period of increased lymphopoietic activity, showing many nodules with rarefied centers and none with germinal centers. He suggests that the findings support his earlier hypothesis for the disappearance of the large number of lymphocytes daily—that they are filtered out by the bone marrow to become the mother cells for erythroblasts.

Removal of lymphoid tissue.—Following careful and extensive

removal of lymphoid tissue from rats and rabbits, Sanders & Florey (66) found no regeneration of lymph glands even when the stimulus to new formation was fully adequate. Further, it was impossible to lower the number of circulating lymphocytes by these means.

Lymphocytes.—Ebert, Sanders & Florey (67), observing lymphocytes within the Clark glass chamber in the rabbit's ear, found them actively motile. No transformations into other types of cells were seen. Lymphocyte cultures in rabbit lymph have been found by Medawar (68) to remain unchanged in the majority of instances but in a few, cells appeared which could be classified as polyblasts. Studying the problem of the fate of lymphocytes and the possibility that polymorphonuclears develop from lymphocytes, Barnes (69) has shown that in the rabbit both cells contain certain enzymes in common: cathepsin, nuclease, amylase, lipase, lysozyme, and adenosinase. In the cat, however, there is no such agreement. Trypsin is present in polymorphonuclears but absent in lymphocytes, while the nuclease and the lipase of the latter are missing in the former.

Sanders, Florey & Barnes (70) found an average daily output of 4×10^9 lymphocytes from the thoracic ducts of cats and rabbits, enough to replace the lymphocyte population of the blood about five times daily. For reasons given in their paper even this figure may be too low. Since operative procedures having little to do with the lymphatic system brought about a great decrease in the number of circulating lymphocytes, the authors could not determine to what extent their quantity was affected by diversion of the thoracic duct's supply of lymphocytes.

In a brief note Meyerhof (71) has reported upon the extraction, from calf mesenteric lymph nodes, of certain substances capable of inducing lymphocytosis when injected into guinea pigs. Similarly prepared extracts from heart, kidney, and liver failed to do this.

Lymph Nodes and Lymphatics in the Processes of Immunity

Developing an earlier demonstration by the present author and Hudack (72) of the formation of antibodies by lymph nodes, Ehrich & Harris (73) have studied the formation of agglutinins and hemolysins in the popliteal nodes of rabbits following subcutaneous injections of typhoid vaccine and sheep red blood cells

in the animals' feet. Changes in the node and in the output of lymphocytes in the efferent lymph gave clues to the type of cell involved. In two to four days after injecting the antigens, antibodies appeared in the efferent lymph and reached their highest titer on the sixth day. A great rise in lymphocyte output in the lymph preceded this and in the node lymphatic hyperplasia occurred following a preliminary infiltration of granulocytes and monocytes. This in turn was followed on the fourth day by the formation of large germinal centers. The antibody titer in lymph followed the increase in the number of lymphocytes but coincided with or preceded the development of Flemming's secondary nodules in the node. The findings point to the lymphocyte as a factor in the formation of antibodies.

The part played by lymphatic channels in the spread of virus infections in the skin of the mouse has recently been stressed by Olitsky & Schlesinger (74), who found the systemic effects following intradermal herpes virus injections enhanced by a previous injection of isotonic solution at the same site, but retarded by injections of hypertonic or irritant solutions. The authors suggest that differences in lymphatic uptake explain the findings: isotonic solutions create a local edema, increase lymph flow, and bring the virus into contact with many more nerve fibers than in inflamed skin in which there may be partial lymphatic blockade.

Lymphatic Drainage and the Treatment of Infected Wounds

Physiologists have pointed out that lymphatics may remain open for a long time after incision (17) and that substances of large molecule and proteins (75) are absorbed from tissues largely by the lymphatics. Trueta & Barnes (76) have used the findings of physiology to explain the excellent results obtained by Trueta's method for the treatment of infected wounds by complete immobilization (77, 78), suggesting that the transport of toxic products by way of the lymphatics will be least in an immobilized limb in which lymph flow is least. To test this supposition, Barnes & Trueta (79) have obstructed lymphatic flow but not the blood circulation in the legs of rabbits, by mass ligations including all the tissues except the femoral blood vessels. Following injections of pyocyaneus organisms into the ligated legs the animals' blood remained sterile, but not so the blood of controls. Rabbits injected with diphtheria toxin into the ligated legs survived longer than

controls, and they were protected from the effects of snake venom of high molecular weight, absorbable probably only by lymphatics, but not from venoms of low molecular weight, which are easily taken up into the blood. By contrast obstruction to the return of venous blood without lymphatic obstruction failed to protect the animals. Complete immobilization of the injected limbs by a plaster spica, instead of ligation, yielded very similar results and protected rabbits from the appearance of the symptoms of tetanus after injection of tetanus toxin, but there was no protection from the effects of strychnine which is absorbed directly into the blood.

Further, they found lymph flow increased during the development of edema, confirming earlier studies of the present author (41), for, as edema formed, both a dye, pontamine sky blue, and pyocyaneus organisms moved faster through the lymphatics than in normal skin. Envelopment of an inflamed part by a closed plaster cast reduced the intensity of the inflammatory edema and greatly decreased lymph flow, which became even less after multiple incisions had been made into the inflamed area to obtain local drainage.

The applications of these findings to the dressing of wounds are obvious. The authors have shown definitely that bacteria and toxins are carried by the lymph, and further that immobilization furnishes a physiological reduction of that flow. In this connection, however, it should be pointed out that there is a slight cutaneous movement of lymph in resting tissues (38, 40, 80). Undoubtedly this cutaneous flow too is obstructed by the plaster spica, for exceedingly small pressure is sufficient. The decrease in lymph flow in a limb immobilized by a plaster cast is not only a physiological one, because of lack of motion, but partly obstructive as far as cutaneous lymph flow is concerned.

The Lymphatics in Shock and in the Alarm Reaction

At present much interest is attached to the role of the lymphatics in the return of fluid to the blood in conditions of shock and hemorrhage. Histamine administered to dogs by McCarrell & Drinker (81) in doses sufficient to lower the blood pressure to 40 to 60 mm. of mercury increased cervical lymph flow 1.1 to 4.9 times the control values. The total lymph protein increased although the concentration became less. Of further interest was the finding that the period of increased protein escape from the blood was tempo-

rary and as the histamine shock continued the lymph flow and its protein content returned to normal. Cope, Brenizer & Polderman (82), stating that man and experimental animals suffering from adrenal cortical insufficiency are in shock, have studied the cervical lymph flow of dogs suffering from this condition. In adrenalectomized dogs they found lymph flow generally decreased but there was no observable correlation between the volume flow of lymph and either the blood pressure or the extracellular fluid volume, the latter being increased in some instances and in others decreased. The plasma volume was found decreased and the blood pressure usually decreased. The lymph protein was increased but the plasma protein was also increased. They conclude that their findings offer direct evidence of increased capillary permeability in the region drained by the lymph collected. One could wish for more conclusive and less irregular data from which to draw this conclusion.

Mayerson (83) found the protein of thoracic duct lymph much increased during the progress of gravity shock in anesthetized dogs suspended in the upright position. A 100 to 300 per cent increase in flow, rich in protein, followed when the animal was put back in a horizontal position. There was no increase in the histamine of lymph or blood.

Selye & Foglia (84) report the appearance of red cells in the sinuses of various lymph glands of the rat and other animals when under the influence of the alarm reaction. The formation of these "hemolymph" nodes was facilitated by intravenous infusions of fluid. The red blood cells appearing in the sinuses were removed after a few days of phagocytosis. The phenomenon is not understood.

Miscellaneous Data on Lymphatic Physiology

Lymph hearts.—As reported by Foglia (85), destruction of the lymph hearts of the toad or frog kills the animal but the presence of a single one maintains life. Paralysis of the posterior lymph hearts by denervation and cauterization of the anterior pair also leads to death. As result, Foglia considers cessation of the contractions of the lymph hearts responsible for death rather than mechanical obstruction of the lymph circulation. Together with Gerschman (86) he has described some of the changes following destruction of the lymph hearts. There was great hemoconcentration, a fall in blood pressure, loss of chloride, sodium, and potassium

from the blood cells, and an increase of potassium in the plasma. The fluids and substances lost to the blood collected in the lymph spaces while water from the exterior collected there too.

Graphic recordings of the pulsations of the posterior lymph hearts of the toad, by Foglia & Braun-Menendez (87), are interpreted to show that, although there is asynchronism between them, the arrhythmia in one repeats itself in the other in such a manner that in any given period of time the frequencies of both are equal. The authors report further (88) an increase in the rate of pulsation with temperature increases and have reported upon the effects of a large number of drugs. The work has been briefly presented in English (89, 90).

Pratt & Reid (91) have brought new evidence to confirm their earlier discovery that lymph hearts of various families of Salientia operate in unison, their spinal centers being axially coordinated. As the paper is chiefly concerned with neurological matter, the reader is referred to it for further details.

"*Lymph time.*"—Copley & Lalich (92), while determining the bleeding time from fingers submerged in water, have observed a whitish flow which they believe is a mixture of lymph, tissue fluid, and plasma, occurring independently of the blood flow or together with it. They termed the duration of the flow "lymph time," to differentiate it from bleeding time which is generally shorter. "Lymph time" was increased by inflation of a pressure cuff on the arm. Since the whitish fluid was obviously a mixture of tissue fluid and plasma skimmed from capillaries and probably contained but little lymph, the term "lymph time" for the phenomenon seems confusing.

Books, monographs, and reviews.—The attention of the reader is directed to the appearance of two larger publications, *Lymphatics, Lymph, and Lymphoid Tissue* (75) by Drinker & Yoffey (already referred to) and *The Lymphatic System* (Lane Medical Lectures) by Drinker (93). Since these works are not confined to recently acquired facts they must not be reviewed here, especially since the newer findings included in them have already been summarized.

Zimmermann (94), studying the development of the lymphatic system in the opossum, revives the old controversy concerning the development of lymphatic endothelium. As space forbids an adequate presentation of his views, the original monograph should

be consulted. Mention should also be made of the eighth volume of the *Arbeiten a. d. Anatomischen Institutes der Kaiserlichen Universität Kyoto* (95), the collection of Japanese papers that appears at intervals from the University of Kyoto. In eighty-two pages the 1941 volume presents twenty articles on such a variety of subjects that they can neither be abstracted here nor even listed. Several reports deal with the concentration of various substances in lymph, others with the physicochemical characteristics of lymph flowing from sarcomatous tissue, others with lymph complement and the flow of lymph through nodes.

The reader is also referred to an excellent review (96) by Freeman & Grodins in the *International Abstract of Surgery* on recent contributions to the study of peripheral blood and lymph circulation.

Intermittency of Capillary Blood Flow and Lymph Formation

Finally, facts of the first importance for an understanding of lymph formation have come to light from experiments not aimed directly at the study of lymphatics. Chambers & Zweifach have observed (97, 98, 99) a rhythmic intermittency in the capillary blood flow through the mesentery of the mouse and frog and in the web of the latter. An outline of their excellent observations cannot be given here; it must suffice to note merely that they describe a characteristic arrangement of vessels which restricts the capillary circulation in resting tissues almost wholly to a very few channels, called by them a-v-capillaries. The great majority of capillaries, true capillaries, exist as offshoots from the a-v-capillaries and, after branching and anastomosing in the tissues, return again to them. In resting tissue, the flow through the a-v-capillaries appears to develop suction forces simultaneously at both ends of the true capillaries, with the result that fluid tends to move out of the tissues into the true capillaries and all along their courses. Intermittently, every few minutes, dilatations of the arterioles occur, which by increasing the blood flow through the a-v-capillaries reverse the flow through the true capillaries, with the result that the tissues become flooded. At this stage a definite flow of lymph begins to appear. In active hyperemia this state continues, and goes far to explain the well known fact that in hyperemia lymph flow is increased (36, 75).

The same authors have made interesting observations (98, 99)

on the intercellular lymphatic and blood capillary cement. The work tends to show that the chemical stability of the intercellular cement, formed by the endothelial cells, controls the permeability of the blood capillary, without taking into account the permeability of the individual cells. The cement of the lymphatic vessels of the mouse mesentery is softer than that of the blood capillaries (98) and during periods of circulatory activity becomes altered before corresponding changes can be noted in the latter.

In regard to the intermittency of blood flow, the present author (100, 101), bringing microscopic amounts of Locke's or Tyrode's solutions into contact with the cutaneous connective tissue of the living mouse, without pressure or under slight pressure and in such a manner that the blood and lymphatic capillaries were not entered directly, found the uptake of fluid strikingly intermittent. These fluids forced into normal skin from a microinjection apparatus at very low pressures also entered it intermittently. In skin deprived of circulation the entrance was continuous but following venous obstruction fluid moved from tissues to the apparatus intermittently. The findings indicated that the interstitial movement of fluid and its absorption from localized regions of tissue were intermittent. Such an intermittency must have corresponding effects upon lymph formation.

Burch, Cohn & Neumann (102, 103) report intermittent volume changes occurring in the tips of fingers, toes, and ears of human subjects. These changes clearly involve accumulation or loss of either extracellular fluid or fluid transported by blood or lymphatics. They believe that the spontaneous fluctuations in volume possibly aid in the distribution and mixing of extravascular fluids and may facilitate the passage of fluid through blood capillary and lymphatic walls.

The implications of these recent findings defy analysis until further data become available but they open up innumerable possibilities for further research. The exchange from blood to lymph in any given, minute, localized region of tissue can no longer be predicted by an understanding of the relationships of the hydrostatic and colloid osmotic pressures within the blood capillaries and the tissue fluids, for mechanical and physiological factors, even psychological ones (102, 103), come into play by their intermittent influence upon the circulation of blood from which all lymph must take its origin.

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The Rockefeller Institute for Medical Research
New York, New York

HEART

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Anatomy and nervous mechanism.—Attempts to relate structure to function are always worthwhile and they continue so particularly with respect to the different muscle bands of which the ventricles are believed to be composed (1). Connective tissue septa separating muscle bundles are described but their existence has not been proved, and the syncytial nature of the heart is so generally accepted that until unequivocal proof of the reality of structurally separate muscle bands in the human heart appears, one cannot reject the concept of the heart as a syncytium. The recent work of others (2, 3) on the conducting system in the human heart has amply shown that many drawings of dissections of the human heart found in standard text books of anatomy are grossly inaccurate and highly imaginative. Attempts to relate to discrete muscle bands definite blood supplies and functions must remain speculative.

A detailed study (4) of the afferent nerve endings in the large arteries and veins is largely of interest to the neuroanatomist. Such endings were found in the arterial ligament, and it was believed that they do not function in fetal life. Other endings, probably terminations of parasympathetic postganglionics, discovered in this region were thought to explain previous observations that the ductus arteriosus contracted on stimulation of the left vagus nerve. The inhibitory effect of acetylcholine on the mammalian heart is so well-known that stimulation obtained after mechanical inhibition on the heart of a small crustacean, *Daphnia magna*, by acetylcholine and physostigmine is of interest (5). The reason for these paradoxical responses, also reported in other invertebrates, is unknown.

Electrical stimulation of the hypothalamus in the anesthetized cat is followed, after a latent period of less than 0.1 sec., by the abrupt increase in activity in sympathetic nerves to the heart and blood vessels (6). This increase ceases with the end of stimulation and is followed by a variable period of time during which all spon-

taneous activity in these nerves is inhibited. The rise in blood pressure may lag one to two seconds after the beginning of the stimulus and continue for several seconds after its termination. The character of the blood pressure response is due to latency and inertia of the sympathetic effectors and not to delay or persistence of neural activity. Multiple pathways connect both sides of the hypothalamus with each sympathetic motor neuron, and the frequency of neuron response depends on the number of pathways excited and the frequency of excitation. Buffer afferents which limit spontaneous sympathetic outflow from medullary centers also moderate outflow occurring from stimulation of the hypothalamus stimulation, and it therefore appears that sympathetic responses from hypothalamic stimulation are mediated through centers in the medulla.

Chemistry and metabolism.—The glycogen content of different parts of the frog heart determined by Best's carmine method and by chemical analyses was found to increase progressively from the sinus venosus to the truncus arteriosus; these findings support the thesis that the glycogen content is directly proportional to the work done by a chamber of the heart and inversely proportional to the intrinsic rhythmicity of the chamber (7). A reversal of the effect of thiamin applied directly to isolated frog ventricle with change in pH was found (8). Between pH 6.0 to 5.2 thiamin was depressant whereas above pH 6.0 it antagonized the action of acetylcholine, while cocarboxylase (the pyrophosphoric ester of thiamin) was depressant to the muscle at all values pH tested. The significance, if any, of these findings in the intact animal is unknown. Dogs fed on a diet deficient in thiamin and prevented from losing weight by tube feeding developed dyspnea and signs suggesting congestive failure, chiefly of the left ventricle (9). Electrocardiograms showed sinus tachycardia, elevation of the RS-T segment, and many ventricular premature beats while examination of the hearts revealed areas of necrosis in the myocardium. The successful outcome of these experiments was attributed to the fact that severe paralyses and marked weight loss were avoided. All abnormal findings disappeared quickly after the administration of thiamin.

Perfusion experiments on the frog heart indicate that the antagonism between calcium and potassium ions is of a limited nature (10) while acute experiments on dogs (11), during which

the concentration of calcium and potassium in the plasma was varied, failed to reveal consistently opposite effects. Increase in plasma calcium caused increases in the intraventricular pressure, the P-R interval, the height of the P wave, and the heart rate while decrease in calcium produced opposite effects on all of these quantities except the heart rate. It is rather surprising that the Q-T interval of the electrocardiogram was not significantly altered by changes in plasma calcium since hyper- and hypocalcemia in man cause well-marked shortening and lengthening, respectively, of this interval. In acute experiments in man, in which 3.0 to 5.0 gm. of calcium chloride was given by vein in periods of three to twenty-one minutes, electrocardiograms showed initial effects (bradycardia, sinus arrhythmia with shifting pacemaker, and heart block) due to vagus stimulation, later evidences of excitation of ventricular muscle (ventricular premature beats and tachycardia), but no striking alterations in the Q-T interval (12). It seems possible that only chronic changes in plasma calcium level are effective in shortening or lengthening electrical systole.

Further studies on blood pyruvate indicate that in normal individuals it varies from 0.56 to 1.1 mg. per cent, that in cardiac patients with congestive failure it is increased (1.2 to 3.4 mg. per cent), but that in heart patients without signs of congestive failure it is normal (13).

The effect of anoxemia without ischemia was studied on isolated heart-lung preparations of dogs in the hope that chemical changes in the myocardium might give some clue to alterations that occur preceding actual disorganization and death of heart muscle due to oxygen lack (14). Muscle analyzed for blood, fat, total carbon dioxide, chlorine, sodium, potassium, calcium, and magnesium showed, after control experiments lasting for one hour, a practically normal electrolyte pattern. Following partial or complete oxygen deficiency, increases in sodium, chlorine, and water and a decrease in potassium suggested extracellular edema without significant changes in the fibers themselves. In other experiments, poisoning of the heart by amytal and by potassium sufficient to produce heart failure produced no significant changes in the electrolytes.

Cardiodynamics.—Cinematographic studies of the tortoise heart show definite increases in the size of the ventricles in pre-

systole which are abolished with ligation of the atria (15). Observations at different heart rates suggest that there is an optimal rate (close to 8 per min.) at which the ventricle does its greatest work and the atrium its least. Interesting high speed photographic studies of the dog heart make possible detailed analyses of the complicated movements of the heart and show, among other things, the existence of distinct inflow and outflow tracts in the right ventricle (16).

Important observations obtained by catheterization of the right atrium in man indicate that normally a pressure gradient of approximately 41 mm. water exists between an arm vein and the right atrium and that this difference tends to disappear in patients with congestive heart failure and high peripheral venous pressures (17). In six normal subjects, determinations of the absolute pressure in the right atrium (accomplished by lateral x-ray localization of the catheter) averaged +37 mm. water.

Using an isolated dog heart and a method by means of which the oxygen consumption could be calculated, other workers found that extra work was more efficiently done with a large venous return to the heart and low peripheral resistance than with a high resistance and a low venous return (18).

Space is not available here for discussion of the means by which the heart and other integral parts of the cardiovascular system compensate (or finally fail), as a result of anoxemia or other changes such as high temperature or humidity, in the physical environment (19, 20). It appears that the degree of anoxia encountered in civil and military aviation at the present time do not damage the normal heart, and the statement "if you can walk, you can fly" is reassuring (21).

Careful studies in several patients during and following recovery from congestive heart failure indicate that stroke volume and cardiac output increase with recovery while the mean peripheral resistance (calculated) drops with improvement (22). In these patients it appears that decrease of cardiac output was one of the factors leading to the development of congestive failure. Studies carried out on patients with circulatory failure in acute infections, such as pneumonia, indicate that reduction in plasma volume is not the cause of the trouble and that the condition differs from traumatic or hemorrhagic shock. It is believed that the heart, as well as the rest of the circulatory system, is damaged

and that the treatment should be directed toward control of the infection (23).

Further evidence that heart failure may be important in the development of a state closely resembling traumatic shock was obtained in a few patients especially following acute myocardial infarction (24). In addition to the usual signs of peripheral circulatory failure, these patients showed high venous pressures or signs of pulmonary congestion. In this connection, studies on dogs (bled until prolonged hypotension appeared then reinjected with the heparinized blood) are of interest (25). In all of the animals, the stroke and minute volume outputs decreased. In some, however, the venous pressure was low and in others it was high, and in the latter group it was suggested that myocardial damage due to poor coronary circulation might explain the low cardiac output. While the calculated total peripheral resistance in these animals usually increased, it was not believed that the development of hemorrhage shock was directly related to changes in the peripheral resistance.

Tourniquets, placed about the four extremities and arranged so each could be inflated to the level of the diastolic pressure and be deflated in sequence after being held under pressure for one minute, were shown to decrease the venous pressure and the cardiac output without altering the arterial pressure significantly in normal subjects and in patients with congestive heart failure (26). This scheme for limiting the venous return to the heart should be valuable in the treatment of left ventricular failure.

Direct and convincing evidence of the benefit to be anticipated from weight reduction in cardiac patients was obtained by a study of several subjects without heart disease before and after weight reduction (27). Weight loss was accompanied by a 16 per cent drop in oxygen consumption, a 30 per cent decrease in cardiac output, and a 24 per cent increase in arteriovenous oxygen difference. It was estimated that the work of the heart in this group was reduced on the average by 35 per cent.

Coronary flow.—Anomalous origin of one or both of the coronary arteries from the pulmonary artery is rare (28). The left coronary vessel is more often affected than the right or the two together; the seventeenth example of the first condition is reported.

Experiments on dogs employing an optically recording orifice meter to measure blood flow from the aorta into a coronary artery

showed that asphyxia and local ischemia increase coronary flow in the absence of pressure changes in the aorta, and since the ratio of flow to aortic pressure (the index of flow) increased more in systole than in diastole, it was concluded that anoxia relaxes the walls of the coronary vessels and alters the dynamics in such a way that there is a reduction of the extravascular forces tending to close the vessels during systole (29).

Further experimental work on dogs indicates that atropine and the xanthine drugs reduce the mortality after ligation of a coronary artery and dilate these vessels. Theobromine sodium acetate was the most effective drug in conscious and lightly anesthetized dogs (30); it reduced the mortality, following ligation of the circumflex branch of the left coronary artery, from 70 per cent, found in the control group, to 23 per cent. In a modified Langendorff preparation with the isolated dog heart (31) xanthine derivatives, particularly aminophylin, metrazol, glucose, calcium chloride, and papaverine hydrochloride were found to cause coronary artery dilitation while preparations of strophanthus and digitalis often have a direct vasoconstrictor action, even in therapeutic doses. Evidence has been accumulated that sudden death in patients after occlusion of a coronary vessel (or in the absence of complete closure) and in dogs following ligation of a coronary artery is due to a lethal reflex resulting in coronary vasoconstriction and ventricular fibrillation. The stimulus arises in the infarct, passes upward through afferent cardiosensory pathways, and returns to the heart over efferent fibers in the vagi (32). Atropine, xanthine drugs, cardiosensory denervation, and even bed rest are believed to interfere with the establishment of this lethal reflex. This theory is of great interest and if confirmed will have far reaching implications.

The question regarding the value of xanthine derivatives in the treatment of coronary artery disease is probably not settled to the satisfaction of everyone but recent evidence favors their use. In an anoxemia test (inhalation of 10 per cent oxygen and 90 per cent nitrogen) aminophyllin, theophyllin with sodium acetate, and theobromine with sodium acetate given by mouth were about equally effective in decreasing RS-T segment deviations in the electrocardiogram in patients with coronary insufficiency (33). Further, by means of a standardized exercise test it was shown that patients with angina pectoris were able to

exercise more before pain appeared and showed much less change in the electrocardiogram following the administration of nitroglycerine, theobromine with sodium acetate, and quinidine sulphate (34). Digitalization made all the patients worse and placebos given as a control for the action of the above mentioned drugs were without effect. Octyl nitrite, a new drug for the treatment of angina pectoris, was found to be somewhat inferior to nitroglycerine (35).

Exercise and anoxemia tests for coronary insufficiency were extensively studied. It appears that the latter test is more specific and safer at sea level (36, 37) than at high elevations (38). In the first studies, only two of 136 normal subjects showed changes in the electrocardiogram previously considered to be significant and of 157 patients with coronary artery disease the test was positive in 77 (49 per cent). No severe reactions were encountered during the tests and it is emphasized that a negative test (no changes in the electrocardiogram) does not exclude coronary artery lesions. At high altitude, the anoxemia test was investigated by age groups in 125 normal subjects (38); in this work, 19.2 per cent showed alterations in the electrocardiograms considered on the basis of previously established criteria to be significant, and furthermore, changes appeared nearly as often in young subjects as in older ones. In 189 tests, 17 untoward reactions occurred and the test was stopped on 15 occasions. Ten per cent oxygen was inhaled in both the studies at sea level and at high elevation but in the latter location, because of the lower partial pressure, this was equivalent to only 8.29 per cent at sea level. It is not clear whether this difference in effective concentration explains the divergent results or not. The effect of graded exercise on the electrocardiogram was studied in patients with and without angina pectoris (39). On the basis of criteria established from tracings of the latter group, it was found that electrocardiographic changes occurred in 56 per cent of the patients with angina pectoris. If pain occurred during the exercise, electrocardiographic deviations appeared in two thirds of the patients whereas only one third of the patients that failed to develop pain showed changes in their tracings.

Determinations of the cardiac output by means of the Wezler-Boeger physical method in several patients following acute coronary occlusions indicate that the output falls rapidly to its lowest

level between the second and third day after the accident, then after the third day, gradually rises until the tenth day when it becomes constant (40).

Optimistic reports concerning attempts to provide collateral circulation for the heart in experimental animals continue to appear (41, 42, 43). Studies on dogs fail to confirm previous reports that the coronary circulation is more adequate following the removal of sympathetic nerves to the heart (44). Except for ligation of a patent ductus arteriosus, procedures to relieve intractable anginal pain, and operations for constrictive pericarditis, surgery of the heart seems to be of little value at the present time (45). The author would, of course, include the repair of the wounds of the heart among the valuable surgical procedures.

Digitalis and other drugs.—The effects of digoxin studied on isolated muscle strips from the hearts of frogs and turtles indicate that both mechanical and electrical systole are invariably shortened by the drug and that this shortening may be the underlying basis of digitalis action (46). On the isolated cat heart, a new material, prepared by replacing the sugar moiety in squills glycoside with two molecules of theophylline per molecule of genin (dimethylxanthine genate), appears to have a wider margin of safety than ouabain, k-strophanthoside, or lanatoside C (47). The expected increase in coronary flow from the theophylline in the compound was not observed. In a study of the effect of strophanthin on blood vessels, it is suggested that the coronary arteries dilate when the heart is damaged but constrict when it is sound (48).

Estimation of the ratio of toxic to therapeutic concentration of purified digitalis glucosides by tests on the isolated papillary muscles of the cat failed to confirm previous observations that the lanatosides, particularly lanatoside C, have a wider margin of safety than glucosides of *Digitalis purpurea* (49). Clinical reports concerning the use of lanatoside C (Cedilanid) indicate that it is effective in all conditions where digitalis may be of value and that it may be advantageous because of its rapid action and constant potency (50, 51).

An electrocardiographic study of the effects of digitalization in normal subjects and in patients with heart disease demonstrated that changes in the electrocardiogram due to the drug are highly inconstant and unpredictable and that the electrocardiogram has no value for the quantitative estimation of digitalis saturation

(52). Electrocardiograms taken before and after standardized exercise in a group of fourteen normal males showed no significant alterations, but when the procedure was repeated after digitalization, distinct depression of the RS-T segment, considered to be definitely abnormal in four instances, appeared in the curves following exertion (53). The significance of these interesting results in the interpretation of exercise tests for angina pectoris is obvious but the suggestion that under these circumstances digitalis may reduce the oxygen supply to the myocardium is highly speculative.

Well-conducted studies (54, 55) of the effects of neosynephrin (*l*-α-hydroxy-β-methylamino-3-hydroxy-ethylbenzene hydrochloride) and synephrin tartrate (*dl*-α-hydroxy-β-methylamino-4-hydroxy-ethylbenzene hydrochloride) in man indicate that the former "differs from epinephrine in its smaller pressor and cardiac potency, its longer duration of action and in its stimulatory effect on some of the parasympathetic effectors, notably those of the cardiac vagus" while "synephrin tartrate is intermediate between epinephrine and neosynephrin in its relative sympathetico-parasympathetico-mimetic action." Paredrine (parahydroxyphenylisopropylamine), another new epinephrine-like compound, is believed to be the most effective preparation given orally for the prevention of cardiac standstill and it produces a more marked and a longer sustained increase in blood pressure than amphetamine (56). It is thought that the pressor effect of the drug is due largely to arteriolar constriction and that it may prove valuable in the treatment of shock.

In several dogs where bradycardia, partial or complete atrioventricular heart block, or ventricular premature beats had been produced by the rapid administration of large doses of digalen, anesthetic doses of pentobarbital caused these findings to disappear, and animals previously anesthetized with this drug were more resistant to digitalis vagal action (57). Since ventricular tachycardia, presumably due to action of digalen on the ventricular muscle, appeared in both types of experiments, the conclusion that pentobarbital depresses the vagus seems to be justified. In a study on the blood pressure, stroke volume, and heart size in the dog during cyclopropane anesthesia, no arrhythmias were encountered (58). Smoking one cigarette was found to lower the alveolar carbon dioxide concentration for ten to thirty minutes

and to increase the heart rate and blood pressure in both smokers and nonsmokers for thirty to sixty minutes (59).

Angiotonin injected into the perfusion medium of a completely isolated cat heart caused constriction of the coronary arteries, decrease in the diastolic volume of the heart, and increases in the oxygen consumption, external work, and the efficiency of the heart (60). Intravenous injection of angiotonin in man produces consistent increases in the blood pressure due to increased peripheral resistance but other findings, such as cardiac output and heart size, were not constantly altered in different studies (61, 62, 63).

Cardiac arrhythmias.—The manner in which ventricular fibrillation developed, as a result of a strong brief direct current shock delivered during the vulnerable period, was studied by three pairs of contiguous electrodes variously oriented on the ventricular surface with respect to the point of stimulation in different tests on the same heart (64). [The ventricles were revived by the counter shock method of Hooker after each test.] Analysis of the electrograms secured suggests that a brief direct current shock produces reentry of impulses that, because of a progressive decrease in refractory period with increase in conduction time, initiates fibrillation. This starts near the site of the stimulus but occurs from repetitive accelerating discharges and not as the result of the stimulus *per se.* This work brings out many other important points and should be consulted by readers interested in this field. Unipolar stimulation, both anodal and cathodal, with direct currents of from 0.5 to 5.0 ma. lasting not over five seconds gave rise to electrical oscillations varying in frequency from 12 to 25 cycles per second and not related to the heart cycle (65). Idioventricular beats and ventricular fibrillation could be produced by both anodal and cathodal polarization, but the former were caused more easily by cathodal while anodal polarization was much more effective in bringing on fibrillation.

In dogs anesthetized with sodium pentobarbital, atrial fibrillation induced by dropping acetyl-β-methylcholine on the wall of the right atrium was stopped within thirty five seconds by intravenous injection of procaine (1 to 6 mg. per kg.), and larger doses of procaine prevented the onset of fibrillation on faradic stimulation of the atrium (66). Similar results were obtained with pontocaine and nupercaine hydrochlorides but in all the experiments the effects were transient.

Observations supporting the suggestion that increased vagal activity occurs following myocardial infarction (32) were made in a group of patients who developed paroxysmal atrial fibrillation or flutter within a few days of such an accident (67). Electrocardiograms showed prolonged P-R intervals in 64 per cent of such patients where tracings were taken soon after or before the onset of circus movement. Data, obtained from 400 autopsied cases, indicates that unless coronary occlusion or congestive failure are present, coronary artery disease is not a cause of atrial fibrillation (68).

Other papers (69 to 73) concerned with arrhythmias cannot be reviewed here.

Pathological physiology and congenital defects.—The rather frequent occurrence of tricuspid stensois with mitral and aortic lesions in patients with chronic rheumatic valvular heart disease is pointed out (74, 75). While such patients usually show prominent peripheral signs of congestive failure (edema, ascites, and hepatomegaly) they may live for years since tricuspid stenosis and regurgitation prevent the high grade and much more serious pulmonary congestion that would appear with mitral stenosis alone. The sudden onset, in patients known to have an aneurism of the aorta, of continuous severe dyspnea with signs of failure of the right ventricle and a long, harsh, continuous murmur along the left sternal edge should suggest the rupture of the aneurism into the pulmonary artery (76).

Ingenious observations made on guinea pig fetuses indicate that the ductus arteriosus can actively close in response to mechanical and other stimuli (77). Inflation of the lungs was found to cause closure of the ductus only when the inflating mixture contained oxygen, and oxygen, given by vein, closed the ductus without inflation of the lungs. Dramatically successful results in the surgical treatment of patent ductus arteriosus complicated by subacute bacterial endarteritis (78, 79) have aroused great interest. Benefit from the ligation is believed to be due to three factors: first, removal from the aorta of the source for infected emboli; second, disappearance of the traumatizing effect of eddies tending to dislodge such emboli into the pulmonary circulation; and finally, increased efficiency of the pulmonary defence mechanisms, due to the reduction of pressure in and velocity of flow through the lungs. Blood for cultures, taken simultaneously from the aorta and pulmonary artery at the time of operation, contained many

more organisms when drawn from the latter vessel; these facts indicated that the lungs play an important role in the removal of infected material from the blood stream and also suggested that some of bacteria in the peripheral circulation enter it through the pulmonary circuit (80).

Successful anastomosis of the pulmonary artery to the left atrium in four dogs produced venous-arterial shunts carrying from 14 to 47 per cent of the flow through the pulmonary artery (81). All of the animals showed cyanosis of the tongue, and in one animal, sacrificed eight months after the operation, subperiosteal proliferative changes in the long bones, similar to hypertrophic osteoarthropathy in humans with congenital heart disease, were found. The amount of blood passing through a venous-arterial shunt in or near the heart can be calculated from data secured by three simple procedures requiring a single venipuncture (82).

Other studies concerned with congenital cardiovascular defects are mainly of clinical interest and cannot be discussed here (83 to 90).

Electrophysiology.—Two electrodes placed very close together on the surface of the ventricle yield electrograms which represent, nearly completely, electrical effects arising in their immediate vicinity. By means of such tracings and simultaneous electrocardiograms, the relative time of electrical activity of a number of points on the surfaces of both ventricles was determined in turtles, dogs, cats, and monkeys (91). Unipolar leads were also taken at the same points by means of one of the paired electrodes, and it was found that the onset of negativity in these curves was practically synchronous with the spike representing surface activity. Leads taken from the endocardial surface of the ventricles showed a deflection indicating much earlier negativity there. The results obtained in this study are what might be expected in the light of previous work. A study of "the sequence of fractionate contraction" at different regions on the surface of the dog's heart was made with the use of special differential electrodes and a simultaneous unipolar lead (92). It is emphasized that the exact time when electrical activation begins in certain regions remains uncertain.

A series of studies in which the important deflections of the QRS complex in leads I and III are explained in terms of electrical effects, supposedly arising from the anterior and posterior

surfaces of the two ventricles, have been published (93 to 97). This work is based entirely on previous experiments in which electrodes were placed on the right and left ventricles of a dog and tracings were obtained after the myocardium in the region of each electrode had been injured by the application of potassium chloride (98). The authors apparently assumed that the electrode on the injured region was entirely indifferent and referred to the curves obtained as dextrocardiograms or levocardiograms when the electrode over uninjured muscle was on the right or the left ventricular surface, respectively. The effect of the activity of one ventricle upon the potential at the surface of the other was entirely ignored. It is not believed that this work is an important contribution to the interpretation of the electrocardiogram.

An ingenious method of obtaining unipolar electrocardiographic leads has been published (99). This method depends upon the possibility of finding on the body surface three points such that the potential of one point is, at every instant, equal to the sum of the potentials of the other two. The authors have not satisfactorily demonstrated that they have succeeded in finding a set of points which fulfills this requirement, but it may be that three such points can be found. It is not surprising to learn that the magnitude of the potential variations produced by the heart is an inverse function of the distance from the heart. We can find nothing in this paper which lends any support to the view that Einthoven's triangle is less valid than he supposed or to the view that Einthoven had any important misconceptions as to the distribution within the body of the electrical currents produced by the heart.

A simplification of the central terminal arrangement for the measurement of the potential variations at a single point on the body is described (100). The usual 5000 ohm resistances are replaced by copper wires of negligible resistance without altering appreciably the form of tracings recorded. A simple scheme for obtaining curves, representing the potential variations of the three extremities one and one half times larger than those usually secured (augmented extremity potentials), is also outlined.

Standard electrocardiograms and precordial leads from one hundred carefully selected normal subjects were analyzed to provide more reliable criteria by which to judge the normality of tracings from subjects thirty to fifty years old (101, 102). Rigid

precautions to insure tracings of the highest technical excellence were taken, and the results of measurements of the form, size, and duration of important waves are recorded in easily accessible tabular form. A simple equation, $Q\text{-}T = \frac{1}{8}(R\text{-}R) + 0.28 \pm 0.05$ seconds, to establish the normal range of the *Q-T* interval for different heart rates is derived. (*R-R*) represents cycle length.

The war has made possible the collection of data of various kinds on large groups of presumably normal young males. Electrocardiograms taken on two thousand aircrew personnel showed, in a number of instances, axis deviation, abnormalities of the *T* wave in lead I and II, and prolongation of the *P-R* and *QRS* intervals beyond the commonly accepted normal limits (103). This study was interpreted to indicate the necessity for more careful selection of aircrew personnel but might also mean that the criteria for judging normality of the electrocardiogram need revision. Studies of the esophageal electrocardiogram indicate that in normal subjects the ventricular complexes in curves taken with the electrode close to the left ventricle are similar to normal precordial leads from the left side of the precordium, and in posterior infarction, changes in the esophageal lead of the same type as those found in precordial leads, in patients with anterior infarction, were found (104).

By means of a vacuum tube amplifier, impulses from the atria in cats and dogs were passed directly in amplified form to the ventricles, thereby producing experimentally, for the first time, electrocardiograms characterized by short *P-R* and correspondingly long *QRS* intervals (105). Furthermore, the supraventricular tachycardia, so characteristic of this condition in man, was reproduced by reversing the amplifier so as to feed impulses from the ventricle back into the atrium. This interesting contribution strongly suggests that an accessory pathway must exist in patients who display tracings of this type. Further studies on electrocardiograms support this view and are opposed to the suggestion that the syndrome is caused by ventricular premature beats arising low down in one of the bundle branches (106). Electrocardiograms from one patient are shown where large doses of quinidine caused reversion of the tracings to normal. In three patients, paroxysms of ventricular and not supraventricular tachycardia were found (107).

Two papers have been published which serve chiefly to demonstrate that the views of American students of electrocardiography

as to the proper interpretation of the human electrocardiograms attributed to bundle branch block are much less divergent than a few years ago (108, 109).

The normal duration of the *Q-T* interval was studied in 1083 men, women, and children (110). A logarithmic type of variation between the *Q-T* interval and the cycle length was found and a general equation, $Q\text{-}T = K \log [10(C+k)]$, was derived. *C* is the cycle length and the values for *K* lie between 0.375 for young men and children and 0.390 for women over 45. *K* was 0.07. It is interesting that in the studies on the *Q-T* interval previously mentioned (101) no significant sex difference was found. During anoxia due to rebreathing or flight in high altitudes, the Bazett index, $Q\text{-}T/\sqrt{(R\text{-}R)}$, is increased, but this occurs only with increase in heart rate (111). It is believed that lack of oxygen somehow prevents the usual shortening of the *Q-T* interval with increase in heart rate. Other studies (112, 113, 114) concerned with the *Q-T* interval cannot be reviewed here.

Electrical effects characteristic of injury to the various muscle spirals are again called upon to explain changes in the electrocardiogram (115, 116). The interpretation given to the observations, upon which all this work is based, is open to serious question and the practice of attaching significance to minimal *RS-T* segment changes, as the authors insist should be done, will be a serious mistake in routine clinical electrocardiography.

Electrocardiograms showing elevation of the *RS-T* segment, followed by sharp terminal inversion of the *T* waves, but without reciprocal changes usually seen in leads I and III associated with infarction, and without *QRS* changes of infarction are presented in a group of patients with chest pain (117). The clinical course was mild in these individuals with good recovery the rule, and it was felt that an acute pericarditis and not coronary occlusion had occurred. Small atypically placed infarcts were not excluded in some of the patients. Since anoxemia, insulin, and vagal stimulation may cause *RS-T* segment depression in the electrocardiogram, it is suggested that such change may be a sign of anaerobic metabolism of the heart (118). Alterations of the *RS-T* segment and *T* wave during or after prolonged attacks of paroxysmal tachycardia are discussed (119).

In studies of the effect of exercise and epinephrine, atropine, mecholyl, and ergotamine tartrate on the *T* waves of the electro-

cardiogram, it was found that cholinergic drugs or factors (carotid sinus pressure) tend to raise the *T* waves and that adrenergic factors tend to lower them (120). The action of mecholyl was exceptional, and increasing the heart rate lowered the *T* waves while the opposite effect occurred with slowing of the heart. Further studies on causes for inversion of the *T* waves in lead II indicate that it may occur in the absence of myocardial disease, especially in nervous individuals with vertically placed hearts (121). Body position, tachycardia, anxiety, and hyperventilation may be important factors.

The effects of ligation of large branches of the pulmonary artery during partial or complete pneumonectomy on the electrocardiogram are shift of the electrical axis of the right (71.4 per cent of patients), the development of deep *S* waves in lead I (50 per cent), and staircase ascent of the *RS-T* segment in lead II (28.6 per cent) (122). These changes tend to appear immediately after the occlusion and often, but not always, disappear within twenty-four hours. No mention of right bundle branch block, which may be present for a time after pulmonary artery embolism, is made. Increase in the amplitude of the *P* waves and miscellaneous changes, such as alterations in amplitude or electrical axis of *QRS* and inversion of the *T* waves in one or more leads, are described during attacks of bronchial asthma (123). The changes may or may not disappear with the end of the paroxysm. The increase in size of the *P* waves was attributed to dilatation of the atria from hypertension in the pulmonary circuit while changes in the ventricular complex were ascribed to "hyperergic reactions" in the coronary vessels. Striking increase in size of the *P* wave in chest lead, CF_2, in a patient with cardiac displacement due to a spontaneous pneumothorax (124), and the value of chest lead, CR_1, in the study of atrial waves in patients with auricular fibrillation are described. In the latter study (125), the circus waves were found to be surprisingly regular and to have an average rate of approximately 400 per minute.

Rather inconstant alterations in the *RS-T* segment and *T* waves in patients and experimental animals with acute pancreatitis are not adequately explained (126). Electrocardiographic studies in patients undergoing fever therapy indicate that high temperature *per se* has no significant effect on the electrical activity of the heart (127). No alterations except increase in size of the *P* waves

in leads II and III and a decrease in the *T* waves immediately after exercise (three rounds of boxing) were found (128). Toxic doses of emetine hydrochloride given by vein to dogs and cats often produced intraventricular block, atrial premature beats, and auricular tachycardia. Bradycardia and atrioventricular heart block was less commonly seen and ventricular premature beats were found only in high grade intoxication (129). This work is of interest as it suggests a means for producing atrial paroxysmal tachycardia in animals. That marked changes in the *T* waves and considerable alteration of the electrical axis of *QRS* occur spontaneously in tracings taken from time to time in rabbits is important information for workers contemplating electrocardiographic studies on these animals (130).

Evidence from two cases that acute myocardial infarction may occur without deviation of the *RS-T* segment in the electrocardiogram is not very convincing (131). It is suggested that subendocardial infarction that does not extend to the epicardium may explain this finding. Other electrocardiographic studies (132, 133, 134, 135) of some interest cannot be reviewed here. Considerable progress has been made in the technique for obtaining fetal electrocardiograms (136, 137, 138, 139). Such tracings have been obtained as early as the fourth month and during the last two months of pregnancy fetal heart sounds and simultaneous electrocardiograms can be recorded in a high percentage of patients.

Heart sounds and murmurs.—The fact that the character of graphic records of the heart sounds depends to a large degree upon the nature of acoustic and electrical setup employed to record them is emphasized in a recent comprehensive study of the normal heart sounds (140), and the suggestion that the third heart sound might arise in the chest wall and not in the heart itself was disproved by experiments on the dog's heart (141).

Strictly speaking, the term systolic gallop rhythm should be used whenever an extra sound in systole produces the characteristic gallop cadence. Some authors exclude systolic clicks (by far the commonest type of extra sound in systole) from this category and employ the term only for a rare type of systolic gallop where the extra sound is heard over the base of the heart. An example of this last variety and four other patients with other types of systolic sounds are reported (142). Of particular interest are sounds appar-

ently of atrial origin occurring with premature beats, probably arising in the atrioventricular node.

A cup-shaped deformity of the right aortic leaflet, situated so as to direct blood regurgitating through an incompetent aortic valve toward the anterior mitral curtain, was found in the hearts of ten patients who had Austin Flint murmurs (143). This finding was believed to support the original opinion of Austin Flint that the diastolic murmur in question was caused by a functional mitral stenosis. Apical diastolic murmurs in five patients, shown by necropsy studies to have dilated hearts but neither mitral stenosis nor high grade aortic insufficiency, were thought to be due to relative mitral stenosis (144).

Sound tracings taken with apparatus capable of recording high pitched sounds show vibrations corresponding to pericardial friction rubs (145). The sounds usually occurred in systole but occasionally were found in presystole indicating that atrial contraction may give rise to friction sounds. Measurements of the time between the peak of the *R* wave of the electrocardiogram and the beginning of the first sound in sound tracings recorded simultaneously in some patients with atrial fibrillation were interpreted to suggest that this interval depended on the length of the preceding diastolic pause (146). From the published curves, it appears that the character of the first sound changes so much that accurate measurement of the time of its onset following short diastoles is impossible. Sound tracings taken from patients following acute coronary occlusion show faint heart sounds and the frequent development of presystolic or protodiastolic gallop sounds (147). These results will not be surprising to any experienced clinician.

Methods of measurement and apparatus.—The application of a previously described method (148) for the estimation of cardiac output from blood pressure and pulse wave velocity to the study of cardiac patients without (149) and with (150) aortic insufficiency is described. This method, like the one employing the ballistocardiograph, can be used on patients with congestive failure. Further studies point out that the ballistocardiographic method is particularly valuable for the repeated study of individual patients during digitalization or to follow changes in the cardiac output that may result from many different causes (151). A ballistocardiograph arranged to record ballistic effects with the subject in an upright position is described (152) and a further discussion of ballistic

theory is presented. The cardiac output in several human subjects, determined by a direct method involving the catheterization of the right atrium, was found to be consistently greater than those obtained from ballistocardiograms taken at nearly the same time (153). The differences, which averaged 18.5 per cent, were felt to be due to inaccuracies in the estimated cross sectional area of the aorta since outputs obtained by the two methods were nearly the same when values for aortic size, obtained by visualization with diodrast, were used in the ballistocardiographic calculations. It was suggested that outputs obtained from ballistocardiograms be arbitrarily increased by 18.5 per cent or that a value for the size of the aorta based on diodrast visualization be used in the calculations.

A detailed discussion of the use of foreign gas methods for the estimation of cardiac output in conditions, such as patent ductus arteriosus where there is recirculation through the lungs, is available (154).

Changes in the size of the chest that occur during ventricular systole and diastole can be recorded by a suitable pneumograph (155). It is claimed that records of this type can be obtained without difficulty and that they are more informative than pulse tracings. A study of pressure changes, occurring in the respiratory passages as a result of the heart beat (the internal pneumocardiogram), was made by means of a bakelite olive attached by rubber tubing to a sensitive crystal microphone and inserted in one nostril (156).

A new type of end-piece for a binaural stethoscope that is claimed to have great amplifying power is described (157). Since the effective pickup area of the unit is large and the air space very small this unit is probably superior to most of those commonly employed. Further studies with the symballophone are reported (158).

A new method for studying the conus arteriosus and the pulmonary artery by means of the x-ray is described (159), and the use of roentgenoscopy as a diagnostic aid in coronary occlusion is advocated (160).

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NERVE AND SYNAPTIC TRANSMISSION

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The present account deals principally with papers on nerve and synaptic transmission which have reached our libraries during the year ending September 1, 1942.

Nerve

The membrane theory.—General physiologists are actively continuing to investigate the role of the cell surface in governing the relations between the interior and the environment of the cell (cf. 1, 2, 3). It may be expected that this work will have its impact on nerve physiology and lead to a considerable revision of the usual explanations of resting and action potentials. The relative concentrations of potassium in axoplasm and in the medium surrounding the nerve fiber have often been considered as a basis for the resting and action potentials, on the assumptions that the nerve membrane is permeable only to potassium and hydrogen ions at rest but to other ions as well during the active phase of the impulse. Recent work on the squid giant axon is in itself sufficient to show the inadequacy of this interpretation. For instance, in contrast with the findings on some other preparations (cf. 4), the change in the resting "membrane" potential with change in the outside potassium concentration is small within the normal range[1] and only approaches the maximal theoretical value for a potassium electrode at abnormally high potassium concentrations (5). Also the ratio of potassium concentrations in the axoplasm and the outside medium is little more than sufficient to account for the resting potential (6, 7), to say nothing of the action potential (see below), even if all the internal potassium is free—a condition unlikely because much of it may be selectively bound by cell proteins and other relatively immobile ions (8, 9). Furthermore, at least under experimental conditions, the axon can gain and lose chloride (10), an ion to which the nerve membrane has been presumed to be impermeable.

[1] It would seem that the axon possesses a reactive mechanism which is able to maintain the normal value of the membrane electromotive force in spite of altered environmental conditions, provided that the latter are not too abnormal (cf. 4a).

In 1939 Hodgkin & Huxley (11) measured the resting and action potentials of the squid axon as recorded between an outside electrode and a microelectrode (sea water-filled pipet) inserted into the axoplasm. Action potentials of 90 mv. were obtained and a tentative determination indicated a resting potential of 50 mv. Curtis & Cole (5, 12) have made additional measurements which confirm these figures. They give reasons for believing that the experimental procedure produced no drastic change of the axon and that the measurements gave a valid measure of the potential difference across the membrane, uncomplicated by the several factors which act when the records are made in the usual way between two surface electrodes. The resting membrane potential averaged 51 mv., with the outside of the axon being positive to the inside. This value was probably about 10 mv. too low because of liquid junction potentials. The spike of the action potential averaged 108 mv. The technical difficulties in these experiments are considerable, and as always the electromotive force developed at the junction of the axoplasm with the salt bridge in contact with it introduces a major uncertainty (cf. 13). Nevertheless, it seems certain that during the passage of an impulse the membrane electromotive force is momentarily reversed in sign; the outside becomes negative with respect to the inside. Thus the action potential can scarcely be explained merely as a simple depolarization of the membrane or abolition of the preexisting resting potential. [Resting membrane potential measurements have now also been made on single muscle fibers (14, 15).]

Determination of the detailed effects of current flow through nerve fibers is made difficult because the greater part of an applied current is usually shunted by surrounding tissues and fluids. A relatively large fraction of a longitudinally applied current, however, passes through the membrane of an isolated giant axon, the central portion of which is placed in an insulator. Use of this type of preparation has enabled Cole & Baker (16) to describe an inductive effect attributable to the axonal membrane, and Guttman & Cole (17) to find that the property of rectification can readily be demonstrated for direct currents. For very weak currents the axon obeys Ohm's law; for stronger currents, still far below threshold, the apparent resistance at the cathode is much less than at the anode.

Cole *et al.* (16 to 19) consider that the simplest equivalent

circuit for a unit area of cell membrane is a capacity of about one microfarad with dielectric loss, shunted by a resistance of several hundred ohms in series with an inductive element of 0.2 henry. Cole (18) has discussed at length the possible physical significance of the capacity and inductance and the degree to which his model explains and fails to explain the known phenomena of nerve excitation and response. He points out that the possible interpretations of an inductive reactance in the nerve membrane are almost unlimited if one considers structures which possess sources of energy.[2] It is precisely for this reason that many would object to a statement that nerve possesses an inductance of 0.2 henry per sq. cm. of surface. The most recent opinion (20), based on experiments with axons in altered ionic environments, is that the structure of the axonal membrane is more complicated than previously assumed.

Some of the thermal and electrical aspects of axonal activity can be explained theoretically on the assumption that the essential structural feature of the nerve membrane consists of two partly expanded lipid monolayers (21). However, neither Danielli nor Cole discuss in detail the essential feature of nerve, namely, the source of electromotive force. Other papers on the mathematical biophysics of nerve may be consulted (22 to 26).

Several other papers have dealt with the effects of ions on nervous structures (27 to 31). Torda (31) notes that although nerves exhibit much more spontaneous firing when soaked in isotonic sodium citrate than when soaked in isotonic sodium chloride, the decrease in the calcium content of the tissue is about the same in each case.

The liberation of potassium by nerve fibers during activity has been much discussed (cf. 32). Now Arnett & Wilde (33) report that excised frog nerves stimulated sixty times a second for one to three hours lose about 11 per cent of their potassium. This observation establishes a liberation of potassium by the myelinated axon during prolonged continuous activity. Faradic stimulation of the isolated frog brain is also said to result in a liberation of electrolytes into the extracellular spaces (34).

Schoepfle & Erlanger (35) have carefully reanalyzed the effect of temperature on the excitability, spike characteristics, and refractory period of medullated fibers. Single axons of the frog

[2] See footnote 1.

phalangeal preparation were tested with shocks similar in wave form to the spike potential of the nerve impulse. Cooling invariably raised the threshold to shocks of this duration—an effect due primarily to a change of excitability, not resistance. Difficult as it is to obtain a true estimate of spike duration, the data clearly indicate a prolongation, particularly of the descending phase, at lowered temperatures. The possible relation of this fact to the enhancement of spinal reflexes by cold (cf. 36) is discussed. The absolutely refractory period considerably outlasts the apparent duration of the spike at low temperatures (10°C.). The actions of polarization, citrate, strychnine, and subthreshold stimuli upon the excitability and the amplitude of the spontaneous oscillations of excitability of axons have also been studied with similar methods (37, 38).

A study of fatigue and refractoriness in frog axons (39) in part confirms previous work (cf. 40). A significant new finding is that delayed supernormality appears several seconds after the end of rapid and prolonged repetitive stimulation. Excitability cycles of such long duration are of great interest with regard to speculations about activity in the central nervous system.

The effects of polarizing currents on the size and duration of the frog A fiber spike have been shown to apply to mammalian A fibers and also to both mammalian and amphibian C fibers (41). The negative after-potential is more sensitive to applied currents than is the spike; it is likewise increased in size and duration by anodal and decreased by cathodal polarization. The late, and possibly also the early, positive after-potentials are similarly affected. Otani (42) has produced additional evidence that during the early part of the refractory period there tends to be a reversal of polar excitability with depressive cathodal influence.

The responses of mammalian motor nerves to slowly rising currents have been studied by several workers (43, 44, 45, 46). Rosenblueth & DelPozo (43) stimulated the blood-perfused sciatic nerve with exponentially rising currents of various time constants. Using as an indicator a muscular response of a given tension, they found that the peak voltage for threshold excitation increased gradually with currents of progressively slower rate of rise. Curves relating the peak voltage to its rate of rise usually consisted of two segments, each of which fits a parabolic equation better than Hill's formula (47). By extrapolation this result suggests that

there is no theoretical limiting slope for the rate of current increase necessary to excite. It is inferred also that the break in the curves connotes two different thresholds with different time constants of accomodation, and consequently that the electric current can stimulate nerve by two independent processes. At the break in the curves the muscle response changes from a quick twitch to a prolonged contraction indicative of repetitive firing. Somewhat similar results have been obtained by Bernhard *et al.* (44), who find "breakdown of accomodation" and repetitive firing with slowly rising currents; this behavior of nerve is considered as a model for sense organ discharges (cf. 48, 49).

Arvanitaki (50) has described and reviewed much of the material on the local, nonpropagated electrical changes which occur in unmyelinated fibers. Bozler (51 to 53) has also described these phenomena in smooth and cardiac muscle. As yet no direct evidence indicates the occurrence of this type of activity in medullated axons (cf. 54) although its presence was inferred by Katz (55) from excitability data.

Structure and function of the osmophilic sheath.—X-ray diffraction studies of nerve lipids, mixtures of lipids, and lipid-protein complexes (56 to 60) have produced data which make possible a more exact interpretation of the structure of the myelin sheath in terms of x-ray measurements (61). The sheath of the vertebrate myelinated axon is composed of concentrically wrapped layers of mixed lipids alternating with thin, possibly unimolecular layers of protein material. The lipid molecules occur as bimolecular leaflets. with their paraffin chains extending radially and their polar groups in the aqueous interfaces, loosely bonded to the protein polar groups. The specific structure of the sheath is relatively insensitive to the action of high temperatures, electrolytes, and detergents, all of which abolish irritability. Therefore, these agents apparently exert their action at a locus which is more critical and metastable than is the bulk of the sheath. It has been pointed out (61) that the parts of the sheath which produce the x-ray diffraction pattern are not necessarily continuous; in the sheath, slabs of "myelin" may be separated by channels of aqueous phase representing submicroscopic or even microscopic faults or crevices. Further studies on the optical properties of nerve fibers (62, 63) also continue to support the current concept of sheath structure.

Those who have dealt with the responses of single myelinated

axons are agreed that the impulse is propagated by segmental jumps (64 to 72, 54), and the studies on isolated axons (54, 68 to 72) indicate that the segmentation in question is due to the interruption of the myelin sheath at the nodes of Ranvier. In experiments with single fibers of the Japanese toad, Tasaki (54) measured the response of a node or nodes in terms of its effect upon the threshold for electrical excitation of a tested node located beyond an intervening blocked node. It was concluded that a fiber responds only at its nodes; that the nodal response is all-or-nothing and involves a considerable decrease of the resistance of the plasma membrane; that there is no local response in the sense of Katz (55); and that the safety factor for the propagation of the impulse is normally about 4.5. These conclusions are in agreement with observations on the action currents recorded from electrodes placed on the nodes and internodes of isolated single fibers (71, 72). Tasaki & Takeuchi (71) find that the action current of a node amounts to 2.0 to 2.6 $\times$ 10^{-9} amps.; it has a brief rising phase of 0.15 msec. and a total duration of 1.5 to 2.0 msec. A node ceases to respond when the action current of the preceding node is reduced by narcosis to one-fifth normal size; this result is in good agreement with the estimations by other methods of the factor of safety for propagation of the impulse.

If the nodes are truly of such importance for the conduction of impulses in peripheral myelinated axons, the mechanism of conduction in heavily myelinated central fibers, which are not usually considered to have typical nodal structures, remains a problem. Recently Grundfest & Campbell (73, 74) have reported very high conduction velocities of up to 160 meters per second in the axons of Flechsig's tract, and have shown a correlation between fiber diameter and conduction velocity for intracentral axons.

Metabolic correlates of nervous activity.—Shanes & Brown (75, 76) have made an interesting study of the relations of metabolism to the resting potential in frog axons. It seems that maintenance of the resting potential depends in part on phosphorylations made possible by the oxidation of pyruvic acid and perhaps also to a limited extent by the anaerobic conversion of phosphopyruvic acid. In skeletal muscle, adenosinetriphosphatase is related to or identical with the protein myosin (77 to 83), and the physical properties of the myosin molecule are reversibly altered by the addition

of adenosinetriphosphate (81) with the subsequent gradual liberation of phosphate. Such a mechanism whereby the properties of an essential structural component of the cell are modified by phosphorylation may be of general occurrence (cf. 84); and Shanes & Brown suggest that the attachment of phosphate to large organic molecules may serve to maintain their structure and alignment in the plasma membrane and cytoplasm. Details of the mechanism whereby the latter would maintain the resting potential are not yet understood but attention is called to an interesting paper of Korr (85). Calcium, which stabilizes the resting potential against reagents and conditions which tend to reduce it (86), also enters the picture; the potential-depressing effects of anoxia and iodoacetate poisoning are counteracted by calcium (87). It is suggested that the effect of added calcium is to shift the equilibria between important cell proteins and phosphate in favor of the phosphorylated state. In this connection it may be significant that calcium acts as an activator for the enzymatic splitting of phosphate from adenosinetriphosphate by myosin (80, 83).

The localization of several enzyme systems in the squid axon has been studied (87a, 87b). Ninety per cent of the succinic dehydrogenase of the giant axon is located in the axoplasm in contrast with cholinesterase (87c, 87d). There is, therefore, no evidence for its concentration in the surface layers. Succinic oxidase has a similar, more or less uniform distribution between axoplasm and sheath layers. Cocarboxylase (diphosphothiamin) is intermediate between cholinesterase and succinic dehydrogenase in that it is definitely but not exclusively concentrated near the sheath—a distribution explicable in terms of its general function in widespread protoplasmic oxidations and its probably specific function in catalyzing the formation of acetic acid for a choline metabolism concentrated near the sheath. All of the enzymes are considerably more concentrated in the head ganglion than in the nerve trunk containing the giant axon.

Nachmansohn *et al.* (88, 89, 90), on the basis of the concentration of cholinesterase near the axon surface, and Beutner & Barnes (91, 92), from model experiments, have come to believe that acetylcholine (ACh) is intrinsically connected with the electrical changes which occur during activity at the neuronal surface, not only at endings but along the entire course of the axon. It is indeed quite plausible that ACh is concerned in some way with the propaga-

tion of the impulse, for in addition to the surface localization of esterase, it is well-known that activity of cholinergic nerves leads to a liberation of an ACh-like substance in amounts which are only quantitatively smaller along the length of axons that at their terminations (93 to 98). Even sensory nerve fibers, which have been thought to be ACh-free, contain small amounts of this substance (95, 99, 99a). However, one must not neglect the unequivocal evidence which shows that a flow of electric current constitutes the essential stimulus for the activation of successive segments of the axon during the propagation of the impulse (54,70,100).

Q_{o_2} values as large as 0.7 were found for the oxygen consumption of different regions of the frog brain shortly after excision (101); these values, higher than most previously reported for the frog, average 40 per cent of those for excised mammalian brain. Freshly excised dorsal root ganglia of rats have a Q_{o_2} of 1.6 and the oxygen consumption per neuron has been calculated to be 4.5×10^{-5} cmm. per hr. at 80 minutes after excision (102). This strikingly high value (cf. 103) indicates that the high respiratory rate of central gray matter may not, after all, be primarily a "synapse maintenance metabolism." An interesting finding is that section of the peripheral intercostal nerves on one side is followed by increased respiratory rates of ganglia excised from both the ipsilateral and the contralateral sides of the body. Oxygen usage of glia (cat filum terminale) is relatively high—three times that of peripheral nerve.

The metabolism of spinal cord tissue has been measured at intervals after asphyxiation of the cord for an hour (104). The oxygen uptake remains nearly normal for six hours and then drops over a period of two days to a level about 45 per cent of normal. These metabolic changes parallel the temporary return of reflex functions in similarly asphyxiated animals (105).

A significant methodological advance is the development of an oxygen electrode which can be used to measure oxygen tensions in brain and other tissues with very little lag (106).

Interaction of adjacent axons.—Katz & Schmitt (107) have previously shown that the passage of an impulse in a single crab fiber is accompanied by a triphasic excitability change, consisting successively of a fall, a rise, and a second fall in an adjacent fiber. These excitability changes were attributed to that part of the action current of the active fiber which penetrates the resting fiber.

The possibility that the excitability changes may have been due to a resistance decrease of the active fiber (108), which would provide a more effective shunt for the test stimulus, was examined (109) by an experiment in which the relative positions of the recording and stimulating electrodes were changed. The results were interpreted to mean that alteration of the testing stimulus by impedance changes in the active fiber is not a significant factor.

The experiments on medullated axons (108, 110, 111) have given different results from those on crab axons in that the approach and passage of impulses in a group of axons produces in adjacent axons a depression of excitability upon which a relative but not absolute excitability increase is superposed. While there is no reason to believe that either set of experimental results is unreliable, it is clear that a rigorous and complete explanation is not yet at hand even for these interaxonal excitability changes, whose interpretation is presumably simple as compared with the electrical aspects of synaptic excitation.

An excellent paper by Arvanitaki (50) has dealt at length with the responses induced in an isolated squid fiber by impulses in an adjacent fiber. She has clarified considerably the phenomena which occur in this particular situation and has discussed their significance for synaptic transmission. Lloyd (112) has shown that the action potentials of synchronously activated muscle fibers directly excite intramuscular nerve fibers of both sensory and motor types; repetitive motor nerve discharges under the influence of physostigmine result at least in part from repetitive responses of the muscle to a single nerve volley (cf. 113, 114,115). Von Brücke & Early (116) found no evidence that dispersed proprioceptive afferent impulses are synchronized as they pass the dorsal spinal ganglia. This is contrary to the findings of Kayser (117).

Synaptic Transmission

The term "synaptic transmission" is often employed to refer not only to the processes which occur when an impulse in a presynaptic fiber excites a postsynaptic neuron or muscle fiber, but also to all the fundamental processes involved in central nervous activity (cf. 118). The available space limits the present discussion to only a few topics.

Analysis of the mechanism of synaptic transmission is impossible in the absence of the most detailed anatomical information

about the investigated systems. Bodian's review on the cytology of synaptic function (119) is, therefore, a most valuable summary. Polyak's book on the retina (120), LeGros Clark's review on the visual systems (121), and Dow's on the cerebellum (122) will also be useful. See also the paper of Silver (123) for the adaptability of the amphibian spinal cord to physiological experimentation.

The Porter phenomenon (cf. 124, 125), in which the ipsilateral respiratory hemiplegia produced by cervical spinal hemisection is abolished by section of the contralateral phrenic nerve, remains one of the most intriguing and possibly one of the most significant puzzles in neurophysiology. It is now found (126) that after spinal hemisection, ipsilateral diaphragmatic contractions are induced by physostigmine, prostigmine, acetylcholine protected by prostigmine, strychnine, vagal section after prostigmine and asphyxia; but none of these agents or conditions is as effective as section of the contralateral phrenic nerve. Species differences (cf. 124) are purely quantitative.

How exclusively do specific neuron circuits and events at synaptic junctions determine central nervous activity? Since fields of current and, presumably, chemical diffusion gradients occur about any active neuron *pari passu*, the excitability of neighboring neurons must be affected even in the absence of synaptic connections with the active cell; axonal interactions (see above) constitute a simple model. The pertinent problem is quantitative: are such effects in the central nervous system sufficiently large to alter significantly the excitability and, therefore, the discharges, of central neurons? Evidence which suggests that they may be has been summarized by Gerard & Libet (127, 128) and by Arvanitaki (50). The extreme localization and precision of activity which characterizes motor discharges and activity in sensory systems (cf. 129, 130) does not necessarily speak against significant action of such "field" effects because in the nervous system neurons are not randomly arranged; those of similar function are grouped in close topographical association. The dorsal root electrotonic potentials, currently studied by Dun (131), offer evidence for electrical interaction between intracentral fibers or neurons even though the specific interpretation of this author (131) will not be universally accepted. Consult also the paper of Barron & Matthews (132).

The synaptic delay is at present (133, 134; cf. 135) defined

purely formally as an interval of time—that in excess of the interval required for passage of an impulse in a full-sized axon from the parent presynaptic fiber to the postsynaptic axon. In several alpha-tempo systems the minimal delay amounts to 0.5 to 1.0 msec., a value now confirmed for relays in the trigeminal system (136, 137). Several time-consuming events, the elucidation of which constitutes a major problem in central nervous physiology, occur during this interval. They may be grouped as follows: (a) conduction of the nerve impulse into the small terminal branches and endings of the presynaptic fiber and (*b*) consequent events in the neuron soma[3] which lead to the initiation of a propagated impulse in the postsynaptic axon.

Neuromyal transmission.—A brief consideration of some of the recent important work on neuromyal transmission is justified by the possible similarity of neuromyal transmission to central synaptic transmission[4], which has been less completely investigated. Eccles *et al.* (141) point out that an understanding of neuromyal transmission requires analysis of: (*a*) the process whereby the motor nerve impulse gives rise to transmitting agents; (*b*) the nature of the transmitter, its mode of action, and subsequent inactivation; (*c*) the local changes set up at the junctional region of the muscle fiber; and (*d*) the initiation of the propagated muscle impulse by these local changes. Steps *c* and *d* have received much consideration; some deductions have been made about *b*; unfortunately, information concerning *a* is not yet available.

As has been mentioned in previous reviews of this series (128, 142, 143), a motor nerve impulse sets up a local change at the motor end-plate. The electrical sign of the effect is called the end-plate potential (e.p.p.). The present conception (115, 144) is that normally the e.p.p. always intervenes between the transmitting agent and the propagated muscle spike; beautiful experiments with the single nerve-muscle fiber preparation of Kuffler (140, 145, 146,

[3] This term was introduced by Lorente de Nó & Graham (138) to refer inclusively to the cell body and the dendrites, but not to the axon of the neuron. "Perikaryon" is sometimes, but less advisably, used with the same connotation.

[4] A striking formal similarity exists between (*a*) electrical changes recorded from near motor end-plates during neuromyal transmission (139, Fig. 8; 140, Figs. 3 and 4); (*b*) microelectrode records from cranial motor nuclei during synaptic transmission (134, Fig. 14); and (*c*) records illustrating the ephaptic excitation of a squid axon by an impulse in a second axon in contact with the first (50, Figs. 2 and 3).

147) show that the e.p.p. is recorded at full size only from a localized region about the end-plate; at this locus the onset of the e.p.p. always precedes the initiation of the propagated muscle impulse which is normally set up in the absence of curare or refractoriness. The e.p.p. has many properties in common with a local cat-electrotonic potential (141, 147, 148, 149, 150). Its time-course is determined by the duration of (*a*) the "active" depolarization caused by the transmitting agent and (*b*) the relatively prolonged "passive" decay of the electrotonic potential built up by the transmitter (141, 147). In the frog, analysis of several types of experiments (147) indicates that the active transmitting agent or process exerts a powerful depolarizing effect for about 2.5 msec. at 18 to 20°C. Feng (151, 152) and Eccles *et al.* (115) exercise considerable caution in their interpretation of the nature of the transmitting agent or process. While such reserve is appropriate in the absence of detailed knowledge of events in the terminal portions of the motor axons or at the nerve-muscle membrane, it is true that the essential transmitter process can be considered as a chemical membrane action due to the acetylcholine cation provided that several, as yet untested, subsidiary assumptions are added to those ordinarily made by chemical protagonists (115).

The essential action of physostigmine is apparently to intensify and prolong the active transmitter process which underlies the e.p.p. (115); that of curare is to shorten and reduce the e.p.p. (115). Curare, therefore, reduces the effectiveness of the transmitting mechanism because an e.p.p. sufficiently reduced by curare is unable to initiate a propagated muscle impulse (140, 141). Thus the action of curare opposes the effect of physostigmine; but at the same time curare does not antagonize the inhibition of cholinesterase by physostigmine (115). However, it seems apparent that pharmacological evidence cannot solve the riddle of junctional transmission in the absence of detailed knowledge concerning the effects of physostigmine, curare, and acetylcholine on the nerve impulse in motor nerve endings.

Action potentials of neuron somas.—Needle electrode records[5]

[5] It has become clear that action potentials of neuron somas are the basis for many of the unitary spike-like potential changes of brief duration which are recorded by microelectrodes in the central nervous system, even perhaps for some which have been attributed to axon spike potentials (154 to 158). Use of extremely small electrodes (3 to 5μ tip) has made it possible to record, in effective isolation,

from cranial motor nuclei (134, 153) have revealed that stimulation of a motoneuron produces, in addition to the nerve impulse which sweeps over the axon, a series of potential changes which must be attributed to an action potential of the soma. The potential changes attributable to the soma are approximately the same whether the motoneuron is stimulated synaptically or antidromically; therefore, an impulse initiated in the axon or at some point on the soma must spread, at least to a certain extent, over the soma. The action potential of the soma is altered and apparently reduced at frequencies of stimulation and levels of asphyxia and anesthesia which do not noticeably affect the impulse in the motor axon. Studies on spinal motoneurons have confirmed these findings (163) and further shown that the action potential of a pool of motoneuron somas, produced in response to stimulation by antidromic impulses in their own axons, may be augmented, or as the case may be, depressed by the central effects of sensory impulses. Even conditioning dorsal root volleys which fail to set up reflex discharges in the axons of tested motoneurons suffice to produce central changes which profoundly alter the soma response. The plausible interpretation is that, in contrast with conduction of the impulse along the normal axon (100, 70), the "factor of safety" for conduction at the soma is small; in fact (retrograde) conduction over the motoneuron soma probably occurs with a decrement[6] which can be augmented or decreased by the consequences of activity in sensory and premotor neurons.

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nerve impulses in single axons (159); but with the somewhat larger electrodes often employed, the rapid triphasic potential change produced by an impulse propagated along an uninjured axon usually produces a relatively small deflection. As Therman (160) described, injury of axons at or near the electrode tip blocks axonal conduction and converts the small deflection, positive-negative-positive at the microelectrode, into a relatively large and monophasic positive potential change, thereby favoring the recording of somewhat dispersed volleys in bundles of axons. Use of this technique, involving admitted injury, has been most useful in determinations of the topography of central tracts, conduction velocities of the constituent axons, synaptic delays at relay stations, etc. (74, 130, 136, 137, 156, 160, 161, 162). If for other purposes it is desired to avoid injury and determine the true form of the potential changes at loci in the gray matter, too much emphasis cannot be placed on the necessity of using small microelectrodes which have a gradual taper to a very sharp point, and which must be very carefully inserted into the tissue.

[6] Decremental conduction may be defined as occurring when the factor of safety for the propagation of an impulse along a conducting structure decreases progressively with distance. The impulse, characterized by an explosive change of

The analyses of Bishop & O'Leary (164, 165), particularly of activity in the lateral geniculate body (165), also indicate the existence of a large somatic action potential. Reference should also be made to the preceding review (128) and to the papers of Libet & Gerard (127) and Rosenblueth & Cannon (166) for recent discussions of the possibility that potential differences across cell layers in the brain, and electrical changes of brain-wave duration, are due to somatic action potentials. The effects of veratrine on synaptic transmission and action potentials, which may be of somatic origin, in the superior cervical ganglion (167) have been investigated and compared with the action of this drug on nerve and muscle (168 to 170).

A most interesting observation is that, subsequent to section of the phrenic nerve, the respiratory discharges into its central stump are reduced; the extent of the deficiency parallels closely the time course of the well-known structural changes of neuron somas in retrograde degeneration (171).

Inhibition.—Marrazzi (172) has extended his studies on adrenergic inhibition of synaptic transmission in the cervical sympathetic ganglion. In the inferior mesenteric ganglion epinephrine likewise inhibits synaptic transmission but it does not influence conduction of impulses in the axons which pass uninterruptedly through the ganglion.

The much discussed possibility of central fibers and endings, in which impulses lead to a lowered excitability of postsynaptic neurons, received support from the discovery that centripetal volleys can produce in spinal motoneurons inhibitory effects of very brief central latency (173, 174); an additional example of such action has been reported (157), and further evidence comes from the work of McCouch *et al.* (175).

Analysis of activity in chains of neurons.—Impulses in fibers of the pyramidal tracts of cats are conducted with a wide range of conduction velocities and exert their action on large spinal interneurons and motoneurons principally via an interpolated nucleus of small cells which is thrown into relatively prolonged reverberative activity (156). Consequently the large neurons are subjected to a relatively slowly developing and statistically smooth excitatory action. It is, therefore, not surprising that the responses

membrane electromotive force, is blocked when the factor of safety drops to less than one; further regions of the conducting structure of course exhibit electrical changes, but only ones of an electrotonic nature.

of these cells occur after a relatively long latency and usually without definite temporal relation to shocks delivered to the pyramidal tracts. The situation stands in striking contrast with activity in the bulbospinal systems where descending volleys are conducted in relatively homogeneous tracts of large axons, which make direct connections with large spinal interneurons and with motoneurons (176). In spite of the conditions making for the delivery of dispersed excitatory effects to spinal neurons, careful analysis, employing microelectrodes and with regard for exact temporal relations and known anatomical facts, makes possible a detailed description of the spinal effects of pyramidal impulses. It is also shown that the three-neuron spinal reflex arc is valuable in the examination of facilitation and inhibition at the interneuronal level, just as the two-neuron arc provides a means of accessing the average excitability of the neurons of a motoneuron pool (177). Lloyd's paper (156) merits the attention of investigators who wish to use action current methods to extend anatomical knowledge.

Observations on the rates of a single motoneuron discharges, as determined or conditioned by the stimulation of central nuclei, can yield much information about the general character of central nervous activity even though such data are not of a nature to permit detailed explanation of excitation and inhibition in terms of the specific properties of neurons (178). Careful studies on hypothalamus-sympathetic neuron systems (178, 179) and on the respiratory center-phrenic motoneuron system (180 to 182) reveal that the motoneuron discharges of these systems resemble, and may be explained in the same general terms as spinal and bulbar somatic reflex activity (134, 135, 183, 184, 185, 186). "The frequency of firing of a sympathetic motor neurone in response to hypothalamic stimulation is determined by the level of excitation maintained by the hypothalamic volleys, the time course of the recovery cycle, and the degree of activity of inhibitory afferents at some critical point between hypothalamus and motor neurone" (178). At the same time, independent of the number of volleys which must summate before the neuron discharges, a sympathetic or a phrenic motoneuron usually fires in response to, or is triggered by, a particular volley after a relatively short and constant latency (178, 180). That normal impulse frequencies in central pathways and motor axons are usually far below the top frequencies at which the structures can be made to

respond has been confirmed by recent studies (179, 180, 187, 188). Experimentally, however, some central neurons fire, presumably in response to synaptic stimulation, at very high frequencies—even over 1000 per sec. (73, 154, 155, 158, 162).

Electrical changes in the cortex and elsewhere have been used for the analysis of cortical activity and for the elucidation of thalamocortical and other connections (cf. 129, 130, 162, 166, 189, 190, 192, 193, 194).

Mathematical biophysicists have presented several theoretical analyses of activity in neuron networks (195 to 199).

Central actions of drugs and other agents.—The actions of acetylcholine, physostigmine, prostigmine, and other drugs on central nervous activity have been further studied in vertebrates, relative to cortical activity (200, 201, 202, 203); to reflex discharges (204 to 208); and on the ganglia of neurogenic invertebrate hearts (209, 210, 211). Bülbring & Burn (208) studied the effects of acetylcholine, physostigmine, and other drugs on the reflexes of dogs in which the cord was perfused by a circulation independent of that supplying the limb muscles serving as indicators of motoneuron discharges. The injection of acetylcholine (0.06 to 1.0 mg.) was followed by motoneuron discharges; physostigmine potentiated the action of acetylcholine; and centripetal volleys in a limb nerve evoked a liberation of acetylcholine into the spinal perfusate. These results were taken (*a*) to mean that injected acetylcholine directly stimulates motoneurons, and (*b*) to be consistent with synaptic transmission by acetylcholine. The authors also mentioned the alternative possibility that acetylcholine merely alters the excitability of neurons to impulses ordinarily subliminal in effect. This suggestion would seem to merit more detailed consideration because motoneurons are subjected to incessant subliminal bombardment by impulses in premotor (afferent and internuncial) axons. The specific action of a drug or other agent on motoneurons can hardly be determined in the absence of most detailed knowledge of its effects on the premotor neuronal systems, in as much as activity in the latter determines the excitability and discharges of the motoneurons. An interesting observation is that acetylcholine affects the knee-jerk and the flexor reflex in opposite ways; apparently either the somatic motoneurons which are involved in the flexor reflex react differently to the drug than do the motoneurons mediating the knee-jerk; or else the activity of

premotor neuronal systems is altered so as to affect the two groups of motoneurons differently.

Gesell *et al.* (212—217) have developed an "acid-neurohumoral theory of nerve cell activation." It is shown that a variety of experimental data are consistent with the inference, based on the well-known pH-activity curve of cholinesterase, that intracellular and extracellular pH should be important in determining the effective action of physiologically deposited acetylcholine.

Acheson *et al.* (218) have shown that the intramuscular injection of tetanus toxin is followed by increased reflex excitability in the ipsilateral part of the spinal cord; apparently the toxin spreads centrally via the motor nerves and alters the excitability of central structures, presumably the motoneurons.

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VISCERAL FUNCTIONS OF THE NERVOUS SYSTEM

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In a general way the differentiation of visceral and somatic functions of the nervous system parallels Pavlov's (1) distinction between neural "activity concerned with the internal world of the organism" and that involved in "the union of the organism with the external world." To a considerable extent these activities are effected, respectively, by the autonomic and the somatic portions of the nervous system.

While the autonomic nervous system is sometimes restricted in reference to the innervation of smooth muscles and glands from ganglia lying outside of the central nervous system, it is now clear that these ganglia are incapable of independent activity when isolated from their central connections (2). Functionally, they appear to constitute efferent relays in the preganglionic outflow from nuclei in the brain stem and spinal cord. Considerable activity, either specific reflex acts or incoherent mass discharge (3), can be effected at the preganglionic level. The full role of the autonomic system in the integration of bodily processes is only exerted, however, through the action of essential supranuclear centers in the medulla, hypothalamus, and cerebral cortex.

Cerebral Cortex

Instances of local activation of adjacent autonomic and somatic effectors support the view that the cortical innervation of autonomic activity serves for providing regional adjustments to somatic functions. Salivary flow resulted from stimulation in man of the mastication field in the lower end of the sensorimotor cortex (4). Lacrimation and pupillary dilatation were elicited from stimulation of the frontal eye field in the chimpanzee (5). In the cat a cortical pupillodilator field was found in the gyrus proreus and genualis, in an area possibly analogous to area 8 of the primate cortex (6, 7). This pupillodilatation is effected entirely by oculomotor inhibition and is mediated by connections passing through the basal forebrain to the mesencephalon (7, 13).

Widespread sweating and vasodilatation following frontal lobotomy in man indicate some more general cerebral influence on the autonomic system, however, the significance of which is not yet clearly apparent (8).

Hypothalamus

From recent studies the hypothalamus appears to stand at the entrance to the lower brain guiding the tempo of the internal realm of our existence with one or more reins to the pituitary gland and others to the autonomic system.

Emotional expression.—While work on the hypothalamus indicates that this structure integrates and possibly reinforces the effector neural impulses controlling some of the sympathetic and motor manifestations of emotion, there is little or no basis for the belief that it governs or even mediates the affective experiences themselves (9, 10). Two cases of "sham rage" in man have been presented (11), one following insulin hypoglycemia and the other following carbon monoxide poisoning. In both cases the involvement of higher centers is stressed and the "sham rage" is believed to be the result of uninhibited hypothalamic discharge. Only fragmentary expressions of rage can be evoked in chronic bulbospinal and mesencephalic cats; compared to exhibitions of anger shown by hypothalamic and decorticate preparations these responses are definitely incomplete (12).

Cardiovascular control.—Excitable hypothalamic regions, the electrical stimulation of which yields responses of purely sympathetic effectors (parasympathectomized iris, nictitating membrane, pilomotor muscles), are similar to those yielding pressor reactions and include the anterior and middle as well as the posterior parts of the hypothalamus (13). Low stimulation frequencies convert pressor to depressor responses and records of electrical activity in the inferior cardiac nerves show this to result from a post-stimulatory depression of tonic cardiovascular innervation (14, 16). Delayed reversals of pressor to depressor responses are due to the liberation of small amounts of epinephrine(15). These studies of response reversals on central stimulation appear to add to rather than detract from the evidence for the specificity of sympathetic centers in the hypothalamus and also in the medulla (14).

Records of the activity in the inferior cardiac and cervical

sympathetic nerves during hypothalamic stimulation (16) show that after a brief latency, stimulation caused an abrupt increase in activity which ceased when stimulation was concluded. For a period thereafter all spontaneous activity was abolished and no evidence of sympathetic after-discharge was encountered. A delay in the rise of blood pressure and its prolongation after stimulation is the result of latency and inertia at the sympathetic effector. The frequency of firing of each efferent neuron is a function of the number of active pathways descending from the hypothalamus and of their frequency of discharge. Interaction of the effects of hypothalamic and buffer nerve stimulation, known to operate at the bulbar level, suggest that hypothalamic responses are mediated through medullary sympathetic centers and not by direct connections with preganglionic neurons.

In a study of the excitability cycle of this system (17), the frequency of discharge of a single cervical sympathetic fiber to constant stimulation of one side of the hypothalamus was examined during periods of test stimulation applied to the opposite side. During testing, summation (increase in the rate of firing) occurred, and at its conclusion, a depression of excitability (decrease in the rate of firing) resulted. The degree of both was a function of the intensity and frequency of the test stimulus.

Direct application of acetylcholine to the tuberal region results in a fall in blood pressure and an increase in the uterus-contracting strength of the blood and spinal fluid. The effect is prevented by atropine and argues for the presence of parasympathetic centers in the hypothalamus (18).

Temperature regulation.—Local heating of the preoptic region of the monkey with high frequency current causes sweating, vasodilatation, and polypnea identical with the integrated response of the animal to an elevated environmental temperature (19). The results are interpreted as indicating that this area contains elements normally activated by rising blood temperature and in turn activating the mechanisms of heat loss.

Bilateral injury to this rostral hypothalamic region in the monkey is followed by an acute postoperative rise in body temperature, which if untreated proceeds to a fatal level (20). This experimental hyperthermia is a result of a central paralysis of the heat loss mechanism together with a maintained or exaggerated activity of the central mechanism for heat conservation. Admin-

istration of pentobarbital during the course of the hyperthermia suppresses the activity of the heat-conservation mechanism (cf. 21) and reduces body temperature to a normal level. The striking efficacy of pentobarbital in reducing experimental hyperthermia warrants its trial in the treatment of neurogenic hyperthermia following injury to the brain in man.

For further consideration of temperature regulation, the reader is referred to the review by Gagge & Herrington in the present volume.

Obesity.—Obesity in the rat has been shown to follow bilateral destruction within a longitudinal zone extending from the ventromedial nuclei backward to the region dorsolateral to the mammillary bodies; it appears to result from interrupting connections arising in the hypothalamus and descending in the brain stem (22). Support for this view is provided by cases of obesity in the dog (23) and man (24); in the dog the importance of interrupting descending connections from the paraventricular nuclei (25) is emphasized (23).

The obese condition may occur in association with or without sexual dystrophy, dwarfism, or diabetes insipidus (22, 24, 26). It does not follow the interruption of hypothalamic pathways to the pituitary, and early hypophysectomy after appropriate hypothalamic lesions does not prevent the appearance of typical obesity (22, 27). Obese animals show a pronounced reduction in running activity (28) and their food intake may or may not exceed that of controls, depending upon the nature of the food supplied (28, 29). Whether the condition simply results from a pathologically enhanced appetite (30) or whether the obese animal's effort to increase food intake and reduce energy expenditure indicates inability to metabolize effectively its available food stores, are alternatives to be further investigated. Evidence has been presented against pronounced lipophilia in the adipose tissue of obese individuals during undernutrition (31).

Gonadotropic activity.—Destruction of the median eminence or extensive interruption of its connections with the hypothalamus has resulted in cessation of ovarian cycles and atrophy of the genitalia in guinea pigs (32) while more distal interruption of the stalk or direct but subtotal injury of the anterior lobe was without effect on estrus cycles (33). Further support for the hypothalamic control of anterior lobe gonadotropic function via the stalk is pro-

vided by the occurrence of ovulation in the rabbit after interruption of a possible alternative pathway in the greater superficial petrosal nerves (34).

This control may be exerted by acetylcholine-like humoral transmission over local vascular channels rather than by direct nervous connections (35), for pseudopregnancy has been shown to follow the application of acetylcholine and prostigmine to the pituitary of the rat, and conversely atropinization of the pituitary prevents the pseudopregnancy induced by stimulation of the uterine cervix. Detailed descriptions of the vascular supply of the human hypophysis (36) and hypothalamus (37) have been presented.

Case reports, in which precocious puberty was associated with hypothalamic involvement in man, have been collected (38), and precocity is attributed to destruction of a posterior hypothalamic mechanism with release of anterior hypothalamic innervation of the pars distalis of the pituitary. On the other hand, precocious sexual and somatic development of male rats, cats, and dogs pinealectomized at an early age and followed to maturity support the view that precocity is associated with involvement of the pineal gland (39).

Neurohypophysial functions.—An atrophy of the supraoptic nuclei and neurohypophysis and degeneration of the neural connections between the two in experimental and hereditary diabetes insipidus have been affirmed (40). In another investigation (41), diabetes insipidus has followed total hypophysectomy plus drastic infringement upon the anterior hypothalamus but has not invariably occurred after hypophysectomy alone or following isolation of the entire hypophysis from the hypothalamus, nor were the supraoptic nuclei always degenerated in the latter preparations. The possibility is considered that there is an antidiuretic elaborating focus in the anterior hypothalamic region (41). Dehydration diminished the antidiuretic potency of the posterior lobe, and the pituitaries of rats with diabetes insipidus induced by stalk section have no antidiuretic effect (42). There was no correlation between these conditions and the number of osmiophilic droplets in the posterior lobe.

Section of the infundibulum in dogs with hypertension of the Goldblatt type was associated with significant decline in arterial pressure in 5 of 8 cases (43). The effect on blood pressure was not

correlated with the degree of resulting polyuria, however, nor did administration of pitressin elevate the reduced pressure to hypertensive levels.

Stimulation of the pituitary stalk in animals with only vascular connections between the head and body led to changes in uterine contractions similar to those induced by injection of pitocin (44). A variety of disorders of pregnancy and labor in guinea pigs with hypothalamic lesions—abortion, prolonged gestation, dystocia, and convulsions resembling those of eclampsia—are considered from the point of view of the hypothalamic control of pituitary and ovarian hormones (45).

The distal termination of nerve fibers of the infundibular stalk are reported (46) to be in three main sites, in the perivascular spaces of the pars nervosa, in the blood vessels of the pars intermedia, and in meningeal terminal corpuscles; these findings indicate "that the main function of the organ (neurohypophysis) must be that of one gigantic perivascular receptive apparatus." Strangely enough, a protein has been isolated from this 'receptor organ' with constant oxytocic, pressor, and diuresis-inhibiting activities (47).

Medulla Oblongata

The bulbar autonomic centers, which in all likelihood constitute the final common pathway through which influences from higher levels reach the preganglionic outflow (16, 17), have been studied particularly during injury or adverse conditions in the past year.

Concussion.—Knowledge of the mechanism of head injury and of the alterations occasioned by it have been greatly advanced by investigation of experimental cerebral concussion (48). This is defined as a direct traumatic paralysis of nervous function without vascular lesion, and is produced by subjecting an animal's head to a sufficiently high rate of change of velocity. Experimental concussion causes transient reflex paralysis of the respiratory and vasomotor mechanism and of other brain stem reflexes. The vasomotor and respiratory centers which are not available to reflexes during concussion are stimulated by the injury. Death from failure of blood pressure may arise from failure of the vasomotor center during severe injury, but the mechanism usually appears identical with that of primary surgical shock, i.e., a peripheral

vasoconstriction with paralysis of the venous side of circulation. The reflex compensatory mechanism then makes inadequate response to the fall in blood pressure.

Neural factors involved in shock have been referred to in reviews on that subject (49, 50).

Increased intracranial tension.—Experimentally increased intracranial tension causes respiratory and cardiovascular alterations, in which the former is more susceptible (51). The systemic venous pressure, in contrast to the arterial pressure, was not significantly altered and the existence of a 'venous pressure center' in the brain appears unlikely. It was not found possible to induce shock by this means.

Anoxia.—During anoxia, increased responses of the nictitating membrane and pilomotor system to hypothalamic, bulbar, and spinal stimulation are in contrast to decreased somatic responses and indicate a fundamental difference in the reactivity of autonomic and somatic centers to this condition (52). Increased pressor responses during anoxia depend, however, on afferent impulses from chemoreceptors in the sinoaortic area. The survival of autonomic responses during general anoxia, like that of other physiological processes, is remarkably longer in young animals than in adults (53). Anoxia in adrenalectomized dogs leads to a fall of blood sugar which is abolished by pancreatectomy; this fact indicates that both the vagoinsulin and sympathoadrenal systems are stimulated by anoxia though the response of the latter overshadows that of the former under normal conditions (54).

The excitability of these contrasting systems has been compared under other conditions, and the vagoinsulin system has been found to predominate during emotional excitement in psychotic patients (55) while the sensitivity of the sympathoadrenal system is increased during experimental thyroid feeding (56) and in magnesium deficiency (57).

Hypertension.—Because complete destruction of the central nervous system lowers the arterial pressure of dogs with renal hypertension to the same level as that of controls similarly treated, it is concluded that the renal pressor hormone is not a peripherally acting vasoconstrictor substance but operates through neural vasomotor mechanisms, setting the center for a high level (58).

Autonomic pathways.—The defects following vascular occlusions destroying the lateral reticular formation of the medulla in

man include an ipsilateral Horner's syndrome, impairment of sweating but not of peripheral vasodilatation in response to heat, and impairment of peripheral vasoconstriction in response to cold but not to other stimuli (59). In the discussion following this paper the intriguing possibility is raised (60) of cutting the sympathetic vasoconstrictor pathway in the medulla for the treatment of Raynaud's disease.

Craniosacral Outflow

Emphasis upon the organization of the parasympathetic system for restricted innervation is provided by the report of ratios of one preganglionic fiber to two postganglionic cells in the ciliary ganglion as compared with one fiber to eleven to seventeen cells in the superior cervical sympathetic ganglion (61).

Iris.—The results of pupillographic studies in reactions to light, darkness, near and distant vision, and in the diagnosis of early lesions of the nervous system have been presented (62, 63). The optimum frequency of stimulation of the parasympathetic supply to the iris is 25 per sec.; the stages of contraction are different when the pre- or postganglionic supply is subjected to prolonged stimulation (64).

Salivary glands.—Salivary flow induced by stimulating the dorsomedial medulla and reticular formation at the level of the seventh and ninth nerves is attributed to excitation of the salivatory nuclei and their efferent fibers in the cat and monkey (65, 66). Since this collection of preganglionic neurons probably supplies the parasympathetic innervation of the entire head, exclusive of the eyeball, the term "salivatory nucleus" seems an understatement of its functional role.

The optimal frequency of excitation of the submaxillary gland through the chorda tympani is 9 per sec.; prolonged stimulation results in changes in secretory rate analogous to the changes in tension of muscle under similar conditions (67). Extensive analysis of electrolyte changes in saliva during pilocarpine and chorda stimulation have been undertaken (68, 69). Other work (70) indicates that the chorda tympani is not a homogeneous nerve but is made up of fibers of different functions—salt fibers, mucin fibers, etc.—which can be excited to different degrees with resulting differences in the composition of saliva. In a monographic account of the electrogram of the submaxillary gland during secretion,

positive and negative components are identified, respectively, with the secretion of water and salts and with the secretion of organic matter (71). Evidence bearing on the neural control of zymogen secretion by the salivary and gastric glands and by the pancreas has been reviewed (72).

Vagal innervation of the heart, stomach, liver, and pancreas.—The disturbance of heart action induced by vagal involvement in a case of syringobulbia has been studied (73), and the nerve supply to the sinus region of the heart is described (74). A remarkable slowing of heart rate (80 to 90 per cent) in the seal during diving is associated with peripheral vasoconstriction and reduction of blood flow in the back musculature, which prevents a fall of blood pressure in the large arteries (75, 76). Factors influencing coronary flow and the clinical course of heart disease, in particular through vasoconstrictor influences exerted over the vagi, are reviewed (77).

In a symposium on gastroduodenal ulcer, neural factors in gastric secretion (78) and motility (79) and in the pathogenesis of ulcer formation (80) are considered. The effect on carbohydrate metabolism of administration of pituitrin, thyroxine, and insulin after vagotomy indicates that the vagi inhibit glycogen mobilization in the liver while the sympathetic accelerates it (81).

In a study of the control of pancreatic secretion (82), stimulation of the vagus resulted in marked increase in the output of enzymes while splanchnic stimulation caused marked diminution in their output. The presence of foodstuffs in the extrinsically denervated duodenum caused an increase in pancreatic enzyme output and rate of secretion, however—a response mediated either by intrinsic nerve plexuses or by some humoral mechanism. Normal carbohydrate metabolism after interruption of the vagi below the diaphragm indicates that in addition to neural innervation some other means of regulation of insulin production exists (83).

Dorsal root efferents.—Stimulation of the peripheral end of the dorsal roots of the spinal cord from T 7 to 9 leads to gastric secretion after a latency of twenty minutes (84). A study of skin temperatures after dorsal root section has failed to reveal a control of peripheral blood vessels which depends upon efferent conduction via the dorsal roots (85).

Bladder and uterus.—Running water through the urethra can produce a bladder contraction through the action of two reflexes, the first a bulbar reflex having its afferent path in the pudic and

efferent in the pelvic nerves, and the second a spinal reflex which has both paths in the pelvic nerves (86). Dilatation of parts of the uterus, most effectively the cervix, augments uterine motility by reflex liberation of oxytocin (44). The intrinsic nerve supply of the immature and adult human uterus has been described (87, 88).

Thoracolumbar Outflow

Efforts to determine effective treatments for hypertension and peripheral vascular disturbances constitute a prominent motive but do not entirely explain the overwhelming concern with vasomotor function that has characterized investigations of the sympathetic system in the past year.

Vascular system and hypertension.—A pronounced fall in blood pressure resulting from the administration of ether to spinal dogs indicates that an important sympathetic outflow for the control of blood pressure occurs between T 6 and 11, but it is not confined to the splanchnic nerve distribution (89). Circulatory collapse in spinal anesthesia occurs principally as a result of accumulation of blood in dilated peripheral vessels (90).

Total paravertebral sympathectomy prevents or abolishes for a time the hypertension following section of the modulator nerves in dogs (91), whereas partial sympathectomies directed toward localized vascular beds are not effective. Circulating pressor substances arising from the kidney are not demonstrably important in the production of this hypertension (92). The return of vascular tone after sympathectomy can best be explained (93) as a heightened responsiveness of the denervated smooth muscle to circulating vasoconstrictor substances, possibly epinephrine and sympathin. After sympathetic denervation, a sensitization of the blood vessels of the leg to acetylcholine has been demonstrated (94).

The results of extensive bilateral paravertebral sympathectomy, in some cases from the stellate to the fifth lumbar ganglion, in hypertensive patients have been reported (95). Lowering of blood pressure in the supine position has varied from a transient to an enduring reduction to normal values. In the upright position lowering was more marked and was present in all cases. Dizziness associated with postural hypotension was troublesome only in the first few months. Hyperactivity of the bowel was only transient. The patients dressed more warmly in cold weather and noticed

excess perspiration in warm weather in those parts still capable of sweating. Such extreme sympathectomy has been demonstrated to be compatible with a relatively normal existence.

Under basal conditions there is no tonic sympathetic control of renal blood flow or urine flow in the dog (96), and measurements of renal blood flow and filtration rate in hypertensive patients before and after sympathectomy reveal no significant change (97, 98). In a study of the intrahepatic circulation in the intact animal (99) vagal stimulation was without effect while excitation of the hepatic plexus caused constriction of the intrahepatic vessels. Investigation of the neural control of pulmonary vasomotor activity in preparations in which the many factors which complicate such a study were controlled has been described (100, 101).

Alterations in cerebral blood flow induced by stimulating the cervical sympathetic are attributed in part to a true constrictor action on the intracranial circulation (102, 103). A part of the innervation of pial, choroid plexus, and brain vessels undergoes degeneration after removal of the superior cervical sympathetic ganglia (104). Associated with this is a temporary fall in cerebrospinal fluid pressure and an increase in the permeability of the blood-cerebrospinal fluid barrier. Local vasospasm induced (105) by stimulation of the pial vessels causes focal cerebral ischemia which should be taken into account in stimulating the cerebral cortex in experiments or at operation. Limited vascular responses to stimulation of accompanying nerves suggest the concept of smooth muscle motor-units (106).

Blood.—A relationship is suggested between overactivity of the sympathetic system and polycythemia, for in dogs with intact sympathetic efferent pathways, modulator nerve section was followed by marked polycythemia and increased blood volume which disappeared after sympathectomy (107). Lesions of the anterior hypothalamic region were associated with a definite and apparently isolated rise in blood urea (108). Accompanying the 40 per cent fall in arterial pressure following acute cord section at C 8 in the cat, there is an increase of plasma volume of 5 per cent (109).

Peripheral vessels.—Spontaneous variations in the volume of the human pinna, finger, and toe, apart from those associated with pulse and respiration, fall into three types of rhythms of de-

creasing frequency and increasing amplitude (alpha, beta, and gamma waves). In phlegmatic or senile subjects the alpha waves are small and constant; in excitable or hypertensive subjects, they are irregular and often of large size (110, 111, 112).

Constrictor reflexes of the hand arteries to cold and loud sound involve only the terminal skin vessels and are without effect on the more proximal vascular supply (113, 114). These observations imply a specificity of response not usually associated with the sympathetic system. Examination of the vascular reactions in the finger to local chilling, indicate an initial reflex constriction upon which is superimposed somewhat later a direct constrictor action of cold (115). Spontaneous rhythms and vasoconstrictor reflexes are absent in the vessels of the forehead skin though the direct action of local cold causes a gradual constriction (116).

Fall in skin temperature and flushing of the distal portion of the extremities on exposure to cold are attributed to arteriolar constriction and capillary dilatation (117). Neither effect is as pronounced in a sympathectomized as in a normally innervated extremity, but very considerable responses still occur after sympathectomy. The alterations are considered a combination of peripheral and central vasomotor adjustments to cold (cf. 115). In responses to heat, a sympathectomized arteriole of the skin is capable of dilating as a result of the direct effect of heat on the vessel itself (118) while capillary dilation (flushing) is under central control exerted through the anterior spinal roots and the sympathetic ganglia.

Because in patients with Raynaud's disease, blanching of the fingers on exposure to cold is not affected by sympathectomy, the disorder is regarded as being primarily a local vascular one (119). Sympathectomy is beneficial in abolishing the aching pain of vasoconstriction (119, 120, 121). Other studies (122) have revealed a definite improvement of capillary circulation following sympathectomy in patients with Raynaud's disease.

Skin resistance and sweating.—By recording changes in skin resistance on stimulating the ventral roots of the spinal cord, the preganglionic sympathetic outflow to the limbs of the cat and monkey has been determined (123). These experiments establish the validity of a method that can be used in the operating room for defining the origin of preganglionic fibers in man. A study of skin

resistance in delimiting areas of sympathectomy and localizing spinal cord lesions is advocated (124); this method offers a number of advantages over sweating tests. In a study of normal individuals with this technique (125), an area of increased sympathetic activity was found in the skin around the eyes, nose, and mouth. This area became restricted to the perioral region during sleep or exposure to cold and dilated upon wakening or exposure to heat; its distribution is compared with patterns of sensory innervation, hair distribution, and incidence of skin cancer.

The rate of sweating from the finger and toe tips of normal resting subjects is very variable; the mean rate from the toe is about half that from the finger (126). Similar values were found in patients with hypertension; these facts suggesting that sympathetic activity is not enhanced in this condition. A reduced and relatively constant rate of sweating exists in senile individuals. Sweating in sympathectomized areas may be evoked by the local application of heat (127) but an intact sympathetic supply is necessary for sweating to occur in a region remote from the heated area. Generalized sweating may be elicited by heating anesthetic areas, presumably through an elevated blood temperature acting on the central nervous system (cf. 19).

Sympathetic supply to the upper extremity.—Recurring differences of opinion concerning the sympathetic outflow to the upper extremity in man hinge around the possibility of aberrant connections (128, 129) or of preganglionic contributions of the first thoracic nerve to the cervicothoracic ganglion (130), which some maintain do not exist (131, 132). The controversy may be resolved by the observation, after chronic interruption of the normal preganglionic outflow to the forepad of the cat [by section of the ventral roots of T 3 to 9 (123)], of changes in skin resistance in the forepad on stimulating the ventral roots of T 1 and 2 (133). This functional reorganization, through the development of pathways from roots which normally do not contribute to the innervation under consideration, possibly by the growth of new collaterals, should be taken into account in planning operations upon the sympathetic system.

Anatomical contributions.—Observations on the location of preganglionic neurons in the lateral column and of possible trophic neurons in the intermediate zone of the spinal cord of man are given (134, 135). Knowledge of the spinal preganglionic out-

flow in the monkey and man has been greatly advanced by a study of the distribution of small medullated fibers in the ventral roots (136). The rami communicantes and sympathetic ganglia have been analyzed with a silver method which differentially stains pre- and postganglionic components (137). Aberrant ganglia are present in the grey rami of the rat, rabbit, and cat, an observation to be considered in the study of these animals after paravertebral sympathectomy (138).

Evidence is presented (139, 140) against a syncytial construction of the vegetative nervous system (141). Contributions have been made to the structure of the cervical sympathetic trunk (142, 143), and the course of sympathetic fibers to the ciliary muscle has been found to be similar to that of pupillodilator fibers (144). The innervation of the adrenal gland has been investigated (145) and reviewed (146), and the surgical anatomy of the superior hypogastric plexus described (147). No correlation has been found between the weight of the celiac ganglion and the presence of essential hypertension in man (148).

Afferents

Although a common sensory mechanism serves both somatic and autonomic motor functions, some afferents, particularly interoceptors, are more directly concerned with autonomic responses than others. Instances of their investigation in the past year may be considered here.

An olfactory-parotid reflex, evoked by introducing citral into the nasal cavity, is present in cases of complete anosmia and the trigeminal and possibly the facial nerves constitute its afferent pathway (149). Salivary flow is predominantly on the side of stimulation but is altered by contralateral cerebral lesions. Application of cold to the nasal mucous membrane of the decerebrate dog decreases coronary flow through reflex inhibition of sympathetic dilator tone; this may explain the occurrence of anginal pain in man upon breathing cold air (150).

Nausea, whether caused by excitation of the semicircular canals or by administration of morphine, is frequently accompanied by generalized contraction of the duodenum, forcing its contents into the stomach (151). Constriction of the retinal arteries induced by vestibular stimulation has been studied after bulbar lesions (152, 153). Audiogenic seizures in the rat are marked by an auto-

nomic and an electrocortical disturbance (154); the auditory stimulus appears to excite autonomic centers in the diencephalon which in turn discharge both peripherally and centrally.

Electrical records of the carotid sinus nerve in the cat reveal small as well as large impulses responding to variations in intrasinusal pressure (155). The small impulses and the pressure reflex of the sinus are abolished by local application of nicotine; this finding indicates the presence of a synapse in the peripheral afferent pathway. Suggestions concerning the surgical treatment of carotid sinus syncope, derived from a study of the anatomy of the sinus nerve in man, are presented (156).

There is no clear evidence that receptors in the root of the superior vena cava are concerned in the cardiac acceleration following increased venous return (Bainbridge reflex), for distension of this region in unanesthetized animals was without effect on heart rate (157). The innervation of the vena cava has been described (158). Anginal attacks induced by distension of the common duct in man are thought to result from reflex constriction of coronary vessels (159). Stimulation of the celiac plexus in dogs evoked vascular and respiratory alterations which were unchanged after section of the vagi but were significantly altered by splanchnic nerve section (160, 161). Similar results are reported for the analogous alterations induced by distending the small intestine (162). Inhibition of the motility of one segment of the intestine by distension of another segment, together with the awareness of distension, are abolished by paravertebral sympathectomy but are not altered by vagotomy (163).

Before concluding, reference should be made to the second edition of "The Autonomic Nervous System" (164) which appeared in the past year. This book will be of interest to the physiologist as well as the surgeon, both for the excellence of its general chapters and for the wealth of original observation it contains on the activity of the autonomic nervous system in man.

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TEMPERATURE REGULATION

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If current events rather than academic considerations determined the tone of a review, this chapter should open with excerpts from Pierre Jean Mauriceau-Beaupré's *Treatise on the Effects and Properties of Cold with a Sketch, Historical and Medical, of the Russian Campaign* (Edinburgh, 1862). Present national activities have greatly increased the number of investigators who are directly concerned with the problems of exposure as recorded by Napoleon's able medical officer at Smolensk a century ago. The immediateness of these problems and the small space allotted to them in previous reviews recommend a considerable emphasis in this review on human adjustment to extreme conditions, and to the biophysical problems of quantifying the thermal stress imposed by unusual exposures.

Hypothermia

Small animals such as the mouse may be rendered hypothermic without drug induction. The mouse exposed to 5° to 7°C. with food eventually develops a rectal temperature below 33°C. (1). During the induction period, which is a matter of many hours, metabolism is enormously increased. The immediate effects of hypothermia, uncomplicated by metabolic exhaustion, are better seen in mice exposed suddenly to −2° to −5°C. Skin temperature (inguinal) falls rapidly. When an inguinal temperature of 20°C. is reached shivering stops. Between 15° and 20°C. consciousness is lost, respiration is reduced, and heart rate becomes erratic at about half the normal value. This condition is reached in two hours. Removal of the mouse to an environment at 15°C. permits maintenance of tissue temperatures from 15° to 20°C. for twenty-four hours, with survival in many animals. It is noted that the immediate influence of hypothermia on tumor growth can only be judged with short induction periods uncomplicated by a pre-

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liminary profound increase in metabolism. In the hypothermic condition there is little variation in tissue temperatures (2).

Hypothermic reactions can be produced in young guinea pigs by the intraperitoneal injection of one to two cc. of clinically active renal extract. Body temperature falls 3° to 5°C. with a return to normal in twenty-four to thirty-six hours. It is suggested that the reaction be used in isolating the hypotensive factor (3).

Mice less than three weeks old are increasingly resistant to ether and chloroform anesthesia when exposed at successively lower body temperatures (4). This is in contrast to the frequently noted increased toxicity of injected hypnotics such as morphine, paraldehyde, and pentobarbital at low body temperatures (5, 6, 7). Peripheral circulatory failure induced in mice by intraperitoneal injections of 50 per cent sucrose is best tolerated by animals in a hypothermic state as contrasted with either normal or hyperthermic animals (8).

A careful study of the order and degree of barbital suppression of the several components of heat regulation in the dog has been made. Doses suitable for surgical procedures were used. Trained dogs were exposed with and without barbital. At the same environmental temperature shivering was delayed two and a half hours and rectal temperature was depressed 2.5° to 3°C. Skin temperature of the ear indicated a long delay in the onset of vasoconstriction. The use of an inductotherm to return the animal to a normal temperature permits a quantitative estimate of the caloric equivalent of the heat deficit incurred (9). A modification of this technique would permit a table of mean tissue temperatures to be prepared and related to the mean gradient in the peripheral tissues of an animal. This would be a convenient tool in considering certain problems in direct calorimetry and in connection with the critical temperature of larger animals at different planes of nutrition (10).

Dogs are unable to withstand severe hypothermia sufficiently long to permit protracted physiological study. In eleven dogs rendered hypothermic by anesthesia induction and ice pack, no animal reaching body temperature below 22°C. survived. For body temperatures between 22° and 25.5°C. the average time of survival was seven hours. Respiratory failure was the most frequent cause of death. Circulatory failure occurred in all dogs avoiding respiratory failure and was associated with cardiac dila-

tation, loss of tone, and anhydremia. Digitalis was found to be most effective in preventing circulatory failure. At 28°C. metabolism was the order of 10.8 kcal. per sq. m. per hr., and at 22°C., 6.5 kcal. per sq. m. per hr. No significant change occurred in blood content of chlorides or carbon dioxide, but a tendency toward hypoglycemia was noted. Blood concentrations showed two marked increases, an initial protective, and a terminal phase in which permeability is increased as in shock (11).

A relatively large literature has accumulated in recent years on hypothermia in humans. The occasion for study of this condition has usually been in connection with cancer therapy. Both clinical and laboratory experience with hyperpyrexia of moderate to severe grade has received long study and now it appears probable that the less common hypothermic condition will receive considerable attention. The impetus for this work came through a recognition of the statistical association between the temperature topography of the body and the frequency of malignancies in various body locations. In general, regions with lower temperatures are less susceptible. This observation in itself would not have provoked serious investigation. The deleterious effect, however, of temperatures below 35°C. on certain embryonal cells, and the recognized effect of lowered temperatures in disorganizing the normal development of such tissues as the incubating chick egg, made it appear probable that the temperature correlation was not an inconsequential one.

Fay and his collaborators were the first to reduce body temperatures by 5° to 10°C. for extended periods. Since then many studies have been made. The general procedure includes an initial period of induction in which temperature regulation is suppressed by evipal or pentothal sodium. The patient is packed with ice bags and body temperature reduced to 32°C. over a period of from four to eight hours. Rectal temperatures are held between 29° and 31°C. for as long as three to five days by adjustments of blankets and cooling agents. Anesthesia is maintained by rectal or stomach instillation of paraldehyde in saline. After the induction period patients are conscious but speech is slow and thick. It is questionable as to whether post-treatment amnesia is a result of sedation or cold. Dehydration occurs as in fever therapy and supportive measures must be taken. Relief of pain and euphoria is the most conspicuous finding in the post-treatment period. It is not agreed

that the treatment is of value in permanently inhibiting tumor growth. Drug addiction, psychoses, and cardiac insufficiency have been suggested as conditions for which the treatment might be suitable (7, 12, 13).

Forty-two patients have been given repeated neurological examinations prior to and following eighty-three periods of refrigeration ranging in length from twenty-four hours to five days. Rectal temperatures were between 23° and 32°C. An increase in the deep reflexes was found when body temperature was between 36° and 29.5°C. Between 37° and 32°C. a period of hyperreflexia appeared which corresponded to the shivering state. Abolition of deep tendon, abdominal, and gag reflexes occurred when temperatures approached 25.5°C. Pupils remained equal, regular, and normal in size throughout but response to light stimuli became progressively more sluggish as temperature fell and was abolished at 25.5°C. Dysarthria began at approximately 34°C. and response to spoken word became practically nil below 26.5°C. Cerebration was progressively delayed with reduction of body temperature and retrograde amnesia was present for the period below 33°C. Although appreciation of pain, heat, and cold was not lost, the symptom of pain, if present before refrigeration, was completely relieved in all but two cases. No permanent alteration of neurologic response was observed (14). Circulation time has been measured with the cyanide method on fourteen patients. Pulmonary circulation is prolonged at internal temperatures of 29°C. A correlation between amount of rectal depression and circulation time was noted only when the femoral vein was the site of injection (15).

Dill & Forbes (16) have made an extended survey of the more strictly physiological factors as seen in mental patients during hypothermia. They define hypothermia as a state of light anesthesia in which certain functions remain moderately effective. Heat production may be above normal as a result of shivering, voluntary activity, or a muscular rigidity of unknown origin. This does not mean that the local metabolism of such organs as brain, liver, or kidney, may not be depressed. Acidosis is indicated by a reduction of alkaline reserve. This effect is unexpected on the basis of observations *in vitro* with decreasing temperature. Arterial oxygen pressure is decreased despite hyperventilation, presumably due to poor lung diffusion. Depletion of carbohydrate reserves may be partly responsible for inhibition of shivering, as

in insulin hypoglycemia, in addition to any direct effect of low temperatures on the nervous system. Respiratory regulation is adequate even at 25°C. A distinction is made in the discussion of blood findings between changes predictable from the effect of temperature in a physicochemical sense and others that reflect physiological responses (15, 16). A decrease in pulse rate not in proportion to the rectal temperature drop has been noted. Irregularities are common and are due to sinus arrhythmia or auricular fibrillation. Vascular constriction is profound and pressure lowered. Normal serum calcium and phosphorous concentrations occur in association with a lengthened electrical systole; these findings suggest cardiac damage rather than a slowing of metabolic processes. Changes in the Q-T interval and abnormalities of the T wave are frequent. Striking leucocytoses as a result of splenic contraction may be seen. Both red and white cells generally increase as a result of hemoconcentration. Cerebral edema occurs (17). The procedure is hazardous and deaths occur (18).

A total reduction of body temperature has been contrasted in certain studies with a local reduction of temperature in accessible areas and such reductions have been maintained for as long as 792 hours (12). No particular physiological studies have been reported in such instances. This procedure, however, has generated interest in the use of cold anesthesia in surgery of the extremities (19). Tourniquets are applied, the limb packed in ice, and surgery begun when sensation is lost. The interruption of venous return prevents the appearance of general exposure effects (20). The striking claims made for this procedure in controlling shock, infection, and postoperative thrombotic and embolic hazards do not concern this review. Such work has led, however, to a reconsideration of the physiological basis for the application of thermal agents to the body surface (21).

At the present time it cannot be said that either the animal experiments in hypothermia or the more hazardous adventures in cold therapy with humans have greatly extended our knowledge of heat regulation. It has been surprising to realize how large the range is in subnormal body temperatures through which the physiologic integration of functions other than temperature regulation may be maintained. This is particularly so when one considers the limited tolerance for supernormal tissue temperatures. It is likely that the real importance of this recent interest in cold exposure will

lie in the attention it will focus on physical temperature *per se* as an analytic technique for use *in vivo*. In any complex system of variables the introduction of a new dimension to which the system is sensitive should afford profound insights. Temperature is naturally not a new factor in homeotherm physiology, but it has been by definition a relatively constant one, and its properties as a variable with substantial range have been confined largely to studies *in vitro*.

Heat Stress

The Fatigue Laboratory has continued its extensive studies of work under conditions of heat stress. The effects of the hot humid climate (29.5° to 32°C. and 85 per cent relative humidity) at Benoit, Alabama, on the performance of work entailing approximately a seven fold increase in metabolic rate are reported. Work at this rate was attempted for a two-hour period. Subjects consisted of members of the research team and white and colored sharecroppers. Some of the members of the research team were unable to continue for more than an hour, and the rest were exhausted at the end of two hours. All of the sharecroppers were able to complete the two-hour period. The white sharecroppers were fit and lean and better in performance than the research team. The negroes were better than the white sharecroppers. Most of the negroes reached an equilibrium with a rectal temperature of about 38.3°C. and a pulse rate of 150 as compared with about 38.9°C. and 170 in the white workers. Sweating was of the order of one to two liters an hour. The negroes sweated less, drank more, and had a higher overall efficiency. Many factors are probably responsible for this practical superiority but the conclusion is reached that the major item was superior cardiovascular function. Final mechanical efficiencies were, with negroes superior, 25.6 and 27.5 per cent. Of twenty-three negroes, two or three were below the comparable white average, and of seven whites, one was better than the average negro. In view of the lower evaporation in the negroes and their higher efficiency, it would be interesting to know whether the lower average skin temperature of the negro group reflected a more efficient distribution of sweating. The differences are small but particularly suggestive in view of the fact that the internal rectal-skin temperature gradient was less in the white than in the negro worker. With comparable heat production in the two groups this would indicate that the circulatory index of the white group was slightly superior to that of the

negro group, at the cost, of course, of an undesirable elevation of twenty beats in cardiac rate. The point is of interest principally in understanding the mechanism by which individuals able to complete a task stabilize at different internal temperatures. Stabilization may occur in two individuals at different absolute levels of internal temperature but with identical circulation indices. It is possible that the equilibrium difference in the two internal temperatures reflects a head of heat stored at the outset through later initiation of physical regulation in the case with poorer adjustment (22, 23).

A large percentage of the recent studies of heat exposure show the increasing effect of current events as expressed in practical utilitarian aims. Men eighteen to forty-five years of age have been studied under hot conditions for a period of four weeks on a seven-and-a-half-hour organized schedule of exercise, rest, and eating. Hot-wet (30.8°C., 87 per cent relative humidity) and hot-dry (38.3°C., 38 per cent relative humidity) atmospheres were used and the principal aim was the compilation of reliable tables of pulse elevations in relation to different schedules of water supply. Full water replacement gave no advantage over one-half replacement during the schedule period. One-half replacement gave some improvement over no-water in hot-wet and pronounced improvement in hot-dry. Saline gave some improvement in hot-wet but a disadvantage in hot-dry. This finding seems unreliable. Lifting in hot-wet produced a higher pulse elevation than marching, and the reverse was true in hot-dry. This is readily understandable after a partitional heat analysis of the two types of environmental thermal stress. The practical conclusion is reached that morning work is desirable and water replacement is desirable for these conditions, but there is a substantial water reserve which is just exceeded by four hours of marching under the hot-dry condition (24, 25). Other authors have briefly discussed salt requirements in the prevention of heat fatigue and exhaustion (26).

In a further study of acute exposure to hot environments in resting subjects it was shown that the initial plasma volume change is quite variable, increasing, decreasing, or remaining static in a manner dependent on the balance between water requisitioned from the tissues and that evaporated. It is recognized that acute and chronic heat exposure may differ substantially in their respective effects on plasma volume (27).

Several studies bear on drug procedures for improvement of

heat tolerance. In work in hot environments adrenal cortical hormone is reported to increase sweat secretion and to achieve stabilization of circulation and the relief of subjective stress at work levels near the individual tolerance. A number of subjects were tested and the individual adjustment data with and without treatments are reported (28).

In a study on rats the thiamin requirements for optimal weight gain at 18.3°C. and 32.8°C. was determined. Optimal growth at fifty-two days in rats maintained at 18.3°C. was produced by 0.8 mg. of thiamin per kg. of food. In a group living at 32.8°C. for the same period the optimal thiamin requirement was 1.2 to 1.6 mg. per kg. of food. Rats with poorest growth in the hot environment were given large doses of thiamin and the temperature increased to 35°C. The animals resumed growth and attained weights equal to the cold room rats. Rats not receiving this supplement lost weight rapidly at 35°C. The basic diet appeared to be adequate except for thiamin. The differences were quite large, and if the findings should be valid for man, they are important (29). Following the administration of glucose or starch to rats in a hot environment, blood sugar levels are elevated over control values. Liver glycogen and the amount of carbohydrate absorbed from the intestine were less than in controls at normal temperatures (30). The varieties of heat sickness have been considered in relation to the symptoms and sequences of events in the several types usually recognized (31).

Central and Peripheral Factors in Regulation

Work continues on the localization of neural centers subserving the separate functions of heat dissipation and heat conservation. Beaton, McKinley, Berry & Ranson (32) have added another excellent study to the long list of important publications from this laboratory. Electrodes oriented by the Horsley-Clarke technique were used for stimulation of the monkey's brain with a low voltage high frequency current. By this method they were able to identify a reactive region in the preoptic area of the telencephalon lying between the anterior commissure and the optic chiasma. The reactive area was found to have a rostrocaudal extent of about 2 mm. and a lateral reach of about 3 mm. to either side of the midline. On heating of this area the animal responded with sweating and polypnea. Urethane was used as an anesthetic and the experi-

ments were carried out at a room temperature of 27°C. The results were credited to heat stimulation and not to severance of any descending pathways from higher centers. The fact that the response disappeared with cessation of heating and could be repeated suggests that it was not due to injury. Tissue destruction was observed to be small. This outcome does not prove that the reactive region contains the final efferent supranuclear neurons. It is quite possible that it contains afferent-like elements capable of activating efferent cell groups more caudally situated. The spread of an area of lesser sensitivity may be due to regions of less compact concentration of sensitive elements, or may illustrate the spread of heat to more specifically reactive points. No associated vasodilation was noted on stimulation. In view of the temperature of the room and the anesthesia, it is pertinent to ask whether or not this should be expected. Peripheral vasodilation may have been present initially. If not, the sudden production of sweating at this neutral temperature could have produced cooling sufficient to maintain tone through the operation of peripheral factors. A new report from Thauer who has published somewhat contrary results in this field earlier is not available because of its foreign source (33).

Many papers continue to appear on peripheral physical phenomena in which both local and central factors are correlated with normal as well as postoperative responses. Gurney & Bunnell (34) found no histological change in sweat glands deprived of their sympathetic nerve supply. The glands, themselves, in spite of severance of their sympathetic nerve supply, are still able to respond to intracutaneous injections of mecholyl and excessive local stimulation by heat. In the human the sympathetic fibers, or motor side of the reflex arc, must be intact for a normal sweating response to occur in a region remote from the heated area. In spite of the absence of sensation in a heated region, a normal sweat response can still occur on other areas. The sensory side of the reflex arc is consequently unnecessary for a normal sweating response to occur following the application of heat. Heated blood acting on the central nervous system is the cause of generalized sweating. Whether the heated blood acts directly on the heat centers or by liberating some hormone which, in turn, stimulates the centers is still uncertain. In a sympathectomized zone, sweating will occur on contact surfaces such as the axilla or antecubital

fossa. It is believed to be due to the stimulation of denervated sweat glands. Sweating tests may serve as an aid in differentiation of disease of anterior horn cells from an involvement of peripheral nerves as an aid to prognosis of recovery of the spinal cord. When sweating is abolished over large areas, other areas are overactive. After cervicodorsal ganglionectomy no evidence is found for regeneration of sympathetic fibers by thermoregulatory or pilocarpine tests up to twenty months, nor after lumbar ganglionectomy by thermoregulatory test up to eight months (35). Hyndman & Wolkin (36) have also considered at length the disagreement over the effect of pilocarpine on the sweat gland following degeneration of its postganglionic neuron. After preganglionic sympathectomy pilocarpine hydrochloride (one-fifth grain hypodermically) will induce sweating up to at least two years after operation. After postganglionic sympathectomy the pilocarpine test performed two months or longer after operation will demonstrate an anhidrosis, the area of which agrees precisely with that of thermoregulatory anhidrosis. When two months have elapsed after operation, the sweat gland, the postganglionic neuron of which has been severed, will not respond to pilocarpine hydrochloride in one-fifth grain doses. The test should be useful in obtaining information concerning the extent to which a sympathectomy is pre- or postganglionic. The two-week period formerly used was thought to be sufficient time for the postganglionic fiber to degenerate. While this is true for the nerve fiber, it may not be true for the end plate. The increased response in the early days following operation is due to the fact that central connections have been interrupted and the existing inhibition of sweating rendered inactive while the end plate remains responsive to chemical stimulation.

The same authors have studied peripheral and central factors in terms of skin temperature and skin appearance on exposure to hot and cold environments. If a nude subject goes from a room at ordinary temperature to one at 10° to 5°C., skin temperature drops markedly; hands, fingers, feet, and toes exhibit greatest changes (10° to 18°C. fall). Central temperature may fall 1° to 2°, followed by shivering. In a young person it will rise again and he will feel warm. If one extremity is sympathectomized, exposure provides a quantitative measure of peripheral response to cold on that side and central response and peripheral response on the

other side. A hand that becomes warmer after cervicodorsal ganglionectomy will remain substantially warmer than its normal mate in a cold environment; both will show arteriolar constriction. A hand that does not become warmer after cervicodorsal ganglionectomy (in persons over fifty years of age) will exhibit the same fall in temperature as its mate; and the temperature of hand and fingers remain equal. Capillary dilatation in response to cold is partially under central control. Pain attributed to vasoconstriction incident to cold is reduced by sympathectomy. Peripheral vascular response to temperature is large and important and is probably an axon reflex mechanism. The central mechanism is important in extreme changes (37). After unilateral cervicodorsal ganglionectomy, patients placed in a heat cabinet occasionally exhibit a definite capillary flushing on the normal side of the face; the denervated side remains pale. The line of demarcation is definite. If heated sufficiently or placed in an inductotherm with or without atropine, the unilateral flushing would always appear. It might be argued that this was inhibition of vasoconstriction rather than active vasodilation. If this were true one would expect flushing on the operated side to occur after ganglionectomy. This has been seen in a mild form in the hands but not in the face. Arteriolar dilatation in heat stress is usually adequate. Capillary dilatation is seen only in extreme cases. It is thought, from operative evidence, that the central control of capillary dilatation is mediated by fibers coursing in the anterior roots of the thoracicolumbar nerves and thence through the sympathetic ganglion. The general conclusion is reached that sudomotor, vasomotor, and pilomotor responses may be governed (*a*) by the end organ: sweat gland, arteriole, and capillary, and pilomotor muscle; (*b*) by a reflex through the spinal cord; (*c*) by central hypothalamic control. Combinations of vasoconstriction and sweating arising from frontal lobe effects on the hypothalamus are not thermoregulatory in character (38).

Duthie & Mackay (39) provide additional evidence that heat (45°C.) applied to one limb under circulatory arrest can give rise to reflex vasodilation in unheated limbs. The afferent impulses may arise from stimulation of nerve endings in the walls of cutaneous blood vessels. Maintenance of constant temperature depends on vasomotor reflexes from skin compensating for moderate variations in environmental temperature. If variations are extreme

a rise or fall in internal temperature will stimulate the hypothalamus. Pain from the heated limb may override vasodilating stimulation to the other limb. Failure of others to find reflex vasodilation may be due to (*a*) pain, (*b*) too low a room temperature, (*c*) previous vasodilation, (*d*) failure to realize that increased heat elimination is not necessarily related to a change in vasomotor tone. It was shown that reflex vasodilation can take place as the result of heating an occluded limb in which thermal sensation is absent. The course of the nerve elements which carry the afferent impulse for reflex vasodilation is unknown, but they remain intact after the vasoconstrictor fibers to the limbs have been interrupted by preganglionic sympathectomy.

Resting blood flow and peripheral vascular responses have been studied in schizophrenic and hypertensive patients. High vasoconstrictor tone was noted in both groups of patients in the hands. No evidence of a lesion in the arterial tree at the periphery was noted in schizophrenia. Forearm and leg flow tended to be normal in schizophrenes and high in hypertensives. Schizophrenes respond with a normal vasodilation under anesthesia (40, 41, 42). A pressure plethysmograph for application to the forearm has been described (43). The action of smoking on skin temperature changes has been reinvestigated. The response is due to nicotine stimulation and not to stimulation incident to inhalation. It is abolished at environmental temperatures above 30°C. (44). Day (45) has continued his studies on the regulation of body temperature in children during sleep. He had previously shown that the child's afternoon sleep was marked by a fall (0.6°) in rectal temperture and a rise and then a fall in skin temperature and rate of evaporation. Inhibition of sweating occurs for a short time with the abrupt cessation of rectal fall. The phenomena are probably due to lowering of heat center sensitivity rather than relaxation.

Gellhorn & Feldman (46, 47) report interesting work on the influence of heat, cold, and experimental fever on the vagoinsulin and sympatheticoadrenal systems. The effect of an increased temperature on the autonomic system is less well known than the effect of cold although it is known that heating of the blood in the carotid artery leads to a stimulation of the vagoinsulin system which is abolished by vagotomy. A comparison of the blood sugar responses of adrenodemedullated-vagotomized, adrenodemedullated, vagotomized, and normal rats exposed to heat and cold indicated

that both systems are stimulated. Cold acts predominantly on the sympatheticoadrenal system and heat predominantly on the vagoinsulin system. Experimental fever produced by intraperitoneal injection of typhoid-paratyphoid vaccine likewise stimulated both systems with a predominant effect on the sympatheticoadrenal. The excitation of the autonomic systems is not the result of fever but due to the vaccine effects on the two systems.

Physical Regulation

Scales of environmental stress.—A precise expression of one phase of the work on heat loss that has been done at the Russell Sage Institute of Pathology, the Pierce Foundation, and the Laboratory of Physiology at the University of Pennsylvania, has been formulated by Gagge (48). Standard operative temperature based on Newton's Law of Cooling is defined as the equivalent environmental temperature with which a warm body (such as a human or animal subject) with surface temperature, T_S, exchanges heat at a standard cooling rate (excluding evaporation effects). Equations are derived from which one may calculate operative temperature when the skin temperature is measured and either the heat loss to the environment or the environmental factors, wall temperature, air temperature, and air movement, are known. It is shown that the loss by evaporative regulation is variant only with operative temperature and invariant with any of the individual physical factors of which it is composed. Use of operative temperature is suggested as a standard reference scale for direct and partitional calorimetry and when it is desired to differentiate between effects of a purely physical nature and those of a physiological nature.

An expression for the standard cooling constant, K_0, is given. A biophysical temperature scale for the human body applicable to all forms of calorimetry may be defined by either of the two relations:

$$T_0 = T_S - (\text{heat loss to the environment})/5.2,$$
$$T = 0.81[T_W] + 0.19[\sqrt{V}\ T_A - (\sqrt{V} - 1)T_S],$$

where heat loss is expressed in kilocalories per square meter per hour and when V is velocity of air movement measured in centimeters per second. T_0 is the operative temperature indicated by the wall or radiant temperature, T_W, the air temperature, T_A, and the observed or assumed mean skin temperature, T_S.

A practical system of units designed to simplify heat calculations under conditions of clothed exposure has been described by Gagge, Burton & Bazett (49). If thermal equilibrium is to be attained without the necessity of major physiological adjustments, the three factors concerned for a balance at an optimal skin temperature are heat production of the body, the insulating value of the clothing, and the environmental temperature. The use of practical units for thermal activity and insulation would provide a uniform system to describe comfortable conditions in relation to the heat exchange of man with his environment.

The proposed thermal activity unit may be defined as 50 kcal. per hour per square meter of the surface area of the individual. This unit is called *one met.* The unit for thermal insulation of clothing is logically the insulation necessary to maintain in comfort a sitting resting subject in a normally ventilated room (air movement, 10 cm. per second) at a temperature of 21°C. and a humidity of less than 50 per cent. This unit of insulation is called *one clo.* Thermal insulation is the resistance offered to flow of heat. It is measured by the ratio of the difference in temperature between two surfaces to the flow of heat per unit area that results. The heat transmitted through the clothing is 38 (76 per cent of 50) kcal. per sq. m. per hr. The total insulation, which is the sum of the insulation of the clothing, I_{Cl}, and that of the ambient air, I_A, is given in metric units by the equation:

$$I_{Cl}+I_A=\frac{33-21}{38}=0.32\frac{\text{degrees C.}}{\text{kcal. per sq. m. per hr.}}.$$

From previous work at the John B. Pierce Laboratory, the insulation of the air in metric units at the air movement cited is

$$0.14\ \frac{\text{degrees C.}}{\text{kcal. per sq. m. per hr.}}.$$

Consequently, the insulation of the clothing, which is equal by definition to *one clo*, is

$$0.32-0.14=0.18\frac{\text{degrees C.}}{\text{kcal. per sq. m. per hr.}}.$$

The *clo* unit is therefore defined as

$$0.18\frac{\text{degrees C.}}{\text{kcal. per sq. m. per hr.}}.$$

In conjunction with operative temperature the system has many applications in the forehanded solution of protective clothing problems of current interest.

Other investigators have given particular attention to the thermal stresses imposed on the body by wind action. Corlette has published extensive tables giving the cooling or heating index and evaporative power of winds (50, 51). Temperature, pressure, relative humidity, and velocity are the factors considered in estimating the caloric absorptive power of an atmosphere. The rather extended computations needed to use the tables have been greatly simplified by Gibson through nomographic methods (52). A general criticism of all such tables is that they choose as a basing point for average skin temperature a single value such as 33°C. in the Corlette tables. A better approximation would be a sliding base from 32° to 37°C. under warm conditions, and 28° to 32°C. under cool conditions. The difference is quantitatively of especial importance in estimating evaporative water demands in heat. A different approach to the problem of providing a wide range instrumental investigation of human heat stresses has been made by Riemerschmid (53). Atmospheric effects are measured by the temperature changes on the surface of a copper sphere enclosing a similar sphere with heat supplied to simulate metabolism. The author has not been in contact with the numerous devices of this general type which have been described in the past. At the present time extrapolations based on adequate biophysical analyses of human heat adjustment in experimental situations can be safely made. Such estimates are more helpful in solving the current problems that motivate such investigations than new instrumentation.

Evaporation.—Newburgh & Johnston (54) have published a recent review which gives an excellent survey of the problem of insensible water loss. The partition between skin and lungs and the use of insensible loss as a measurement of heat production is discussed in detail. Other topics covered are the use of insensible loss in the quantitative measure of water exchange and the relationship of water content of the body to insensible water loss. Attention is given environmental effects including temperature, humidity, air velocity, and clothing.

Papers not available for the above review are limited in number. Lippmann (55) has checked the Benedict data for the climate

of Sydney, Australia, and found that 80 per cent of all normal cases showed values for insensible perspiration within plus or minus 10 per cent of predicted values. The remainder gave values 10 to 20 per cent lower than predictions. Findings were not considered reliable if the basal rate exceeded 1800 kcal. per day or if the insensible loss exceeded 40 gm. per hour. The total average loss without correction was 19.5 gm. per sq. m. Daytime sleep decreased insensible perspiration by about 15 per cent. Erythema (produced by ultraviolet radiation) decreased the loss greatly by means of a local effect. Pyramidon has no effect. Reliable results in experiments on insensible loss can be obtained only if the skin temperature is kept normal and constant, as slight cooling (with decrease in skin temperature of 2° or 3°C.) decreases the rate by 15 to 20 per cent. Control of skin temperature is therefore absolutely essential. The method is considered to be supplementary to and often an improvement on the determination of the basal metabolism. Burch & Sodeman (56) have made observations with controlled atmospheric conditions (varying from 24°C. and 50 per cent relative humidity to 35°C. and 75 per cent relative humidity). These were done in winter and spring to observe the rates of water loss from localized surfaces of the body including segments of the head, trunk, and upper and lower extremities. In normal subjects a typical pattern was found. The rate of water loss per unit time per unit area was greatest on the finger tips and palms, toe tips and soles of feet, and head—in that order. The rate was lowest on the trunk, leg, thigh, and forearm. The loss was intermediate in the axillae. A more or less uniform increase in loss occurred in all areas following a change in the atmosphere from comfortable to hot. In diseased individuals somewhat different observations were made. Scleroderma caused compensatory increases in low sweating areas if high sweating areas became impaired. A patient who was markedly uncomfortable in a hot atmosphere failed to respond normally to increased environmental temperature. In a second study (57) members of the same group measured the water loss from the finger tips and toe tips of normal senile patients and patients with arterial hypertension. A very marked increase in loss was found in measurements made in the spring as contrasted with those in the summer months, despite a controlled room temperature of 24°C. Rates for the finger in the spring averaged 3.59 mg. per sq. cm. per 15 min. as against 2.10

for the hot months. The authors note that the sweat mechanism is more sensitive in the summer period but that this is contrary to their local areal finding. In view of the fact that palmar and finger tip sweating is not thermal in character, this is not necessarily a contradiction. Palmar surfaces of finger were notably moist when rate of water loss exceeded 5 mg. or more. Mean rate in hypertension was about the same as in normal subjects, but the rate in senility was reduced, presumably due to partial atrophy and decreased function. It may be that sympathetic tone is raised in hypertensives only under conditions of stress. Rates were also less variable from period to period in older patients. After the lower thoracic and lumbar sympathetic nerves were cut and celiac and aortic renal ganglia excised in hypertensives, a decrease of as much as 50 per cent in toe loss and a comparable increase in finger loss was often noted. A further study of the same areas as well as of the pinna was made (58). Pinson (59) has studied the loss of water with the sweat glands inactivated. Subjects were examined at 28°C. Two symmetrical areas were compared, one of which had the sweat gland inactivated by the Kuno technique. The skin was covered with 5 per cent formaldehyde and a copper anode applied. (A current of 3 ma. per sq. cm. for ten to fifteen minutes constituted a treatment.) Three treatments suppressed activity for two to four weeks. General exposure to heat produced an increase in water loss in the leg so treated. Peripheral blood flow as induced by histamine or pituitrin distal to the moisture absorbing skin capsule did not influence the rate although skin temperature rose slightly. Cutaneous insensible perspiration doubled in rate with a skin increase of 10°C. Rate of blood flow had no effect except as it altered skin temperature. Increased vapor tension decreased the rate. The rate of insensible loss at a skin temperature of 26°C. is approximately 0.01 mg. per sq. cm. per minute. Winslow, Herrington & Nelbach (60) measured surface moisture on the mucous membrane of the oral mucosa by an absorption technique. This study of local moisture was directed at detecting the drying effect on the mucosa of respiration in atmospheres of various temperatures and humidities. The drying effect of the respired air is a function of the absolute vapor pressure. With a vapor pressure of 10.2 mm. Hg. and above, the surface of the oral mucosa is relatively moist but the moisture on the surface varies from a moderately high to a very high value, de-

pending on variations in both temperature and humidity. These variations are attributed to physiological changes in the vascular system of the mucosa. With vapor pressures below 10.2 mm. Hg., there is a marked drying of the oral mucosa; the moisture present on its surface is about half that observed at higher vapor pressures. Marked drying of the mucosa must, therefore, occur at air temperatures below 11.6°C. with any moisture content; at a temperature of 15.6°C. with less than 77 per cent relative humidity; at a temperature of 21.1°C. with less than 54 per cent relative humidity; and at a temperature of 26.7°C. with less than 39 per cent relative humidity. Whether such drying is harmful or not has not been clearly demonstrated. If it is harmful, however, exposure to outdoor air in winter must have as serious an effect as that exerted by dry indoor air. If the effect is to be controlled under normal indoor conditions (21.1°C.) over 50 per cent relative humidity must be maintained. Barbour (61) has summarized his long series of studies on the control of water movement in response to environmental temperature. Particular attention is again given to the heat conservation properties of vapor pressure changes incident to water shifts as they affect the availability of fluid for insensible loss and sweating.

Partitional calorimetry.—It has been shown that the influence of markedly different radiant and air temperatures is accurately represented by the concept of operative temperature. Physiological responses are in accord with the integrated effect of radiant and air temperature and the degree of air movement (62). The technique has been extended to the working subject and heat loss constants derived for the several avenues of heat loss under working conditions. Heat regulation in the trained subject is remarkably effective. Over a range of operative temperature from 12° to 30°C. with moderate humidities the body maintains a mean skin temperature at a work rate of 300 kcal. per hr. which is lower than that of the resting subject in the same environment. At the lower operative temperatures a skin temperature is subjectively comfortable which would be intolerable in the resting subject. Peripheral thermal sensation is markedly influenced by the gradient beneath the skin as well as the actual surface temperature (63). A convenient method for the continuous recording of high rates of oxygen consumption has been described (64). The method of estimating peripheral blood flow in terms of the circulation index

developed in conjunction with partitional methods has been applied by Stewart and co-workers (65). It must be emphasized that this technique is valid only in equilibrium states in the absence of a complete set of partitional measurements.

Chemical Regulation and Slow Adjustments to Temperature

The distinction between emergency and long term adaptation to thermal stress in the neural, glandular, and tissue components of the complete mechanism of temperature adjustment is now conventional. Investigation proceeds principally in the direction of quantification and separation. Ring (66) has shown that following exposure to cold for three weeks, basal heat production is increased only 10 to 15 per cent in rats. This increment cannot be sufficient to account for the poor tolerance of the thyroidectomized animal. Thyroxine potentiates the calorigenic response to epinephrine which is the protective factor. Bernstein (67) found a seasonal variation in thyroid epithelial cell height in sixty- to ninety-day old rats, and also in the distribution of adrenal cortical sudanophil substance. The thyroid index rigidly follows a seasonal curve while the adrenal index is more likely to vary with thermal changes which may be considered normal. Laboratory temperature did not vary. Epithelial cell height is inversely related to experimentally altered temperature whereas the distribution of cortical sudanophilic material is directly related. These variations are reversible provided no tissue damage occurs. The occurrence of a seasonal change of a type that can be produced by an experimentally altered temperature despite a controlled cage temperature is difficult to explain. An activity cycle correlated with seasonal light changes is a possible factor.

Lee (68) has studied the effect of previous environmental temperature on basal heat production measured at 28°C. in the rabbit. There has been some question as to whether the adaptation in basal rate after cold or hot exposure is peculiar to small animals. The metabolic level of the rabbit was dependent on the temperature at which they had been living prior to the measurements at 28°C. and the length of exposure at this temperature. The major metabolic adjustment to an increase in temperature toward thermic neutrality took place in two to three weeks but further adjustment continued up to at least two months. Adjustment to lower tempera-

tures was less rapid. The maximum range in deviations from the basal level in metabolism measured at 28°C. amounted to 26.1 per cent in a group of five rabbits maintained at 17° and then 31°C. When moved from 10°C. to 29°C. the range of deviation was 17 per cent. Metabolism varied inversely with the change in previous temperature. Hence, when the rabbit is used in studies of the effect of a superimposed condition the previous habitat temperature should be maintained at 28° to 29°C. for three weeks prior to measurement.

Herrington & Nelbach (69) have studied the effect of increased metabolism on the pattern of glandular weights in the rat. Rats maintained at 28°C. for 210 days provided a control pattern. Metabolism was increased in other groups by four techniques, maintenance for 210 days at 18°C., at 35°C., at 28°C. with thyroid feeding, and at 28°C. with disturbance by noise, vibration, and high air movement for ten minutes in each hour. When body, organ, and gland weights of each group are plotted as percentages of the corresponding weights of the 28°C. temperature group, two patterns are discovered, one typical of temperature effects (18° and 35°C.) and the other typical of stimulation effects (thyroid feeding and disturbance). Small testicular and large prostatic and pituitary weights were the results of high or low temperature; the animals of the 35°C. temperature group had, in addition, very small adrenals. This endocrine pattern of the 35°C. group is distorted in a manner normally associated with an age effect. The most characteristic differences in the patterns of the disturbed and the thyroid groups are the small pituitaries of the former and the large hearts of the latter. Decreased weights of the pituitary, increased weights of the adrenals, and decreased weights of the testes of these groups in relation to the 28°C. temperature group suggests an androgenic effect of adrenal cortical origin. Thermoregulatory, neurogenic, and pharmacological means of chronic elevation of metabolism produce consistently different patterns of glandular weights. The method is of value in studying the total response of the intact animal. The tensility characteristics of the aortae from the animals of this study have been reported. Chronic heat or cold exposure produces profound differences in aortic tensility. High temperatures have an age-mimetic effect (70).

Hardy, Milhorat & DuBois (71) have confirmed an earlier report which presented evidence of immediate chemical regulation

in women. Measurements of basal rate in women are compared with those of men using a calorimetric procedure. The basal heat production of women in the cold zone was about 35.0 kcal. per sq.m. per hr. and approximated that of men. In the warmer zone the women showed a marked drop in basal metabolism which averaged 30.9 kcal. per sq. m. per hr. at 31°C. This indicates a type of regulation which is not found in men. In women the fall in metabolism was concomitant with a marked decrease in heat loss in the comfort zone reaching at 31.5°C. as low a level as 28.5 kcal. per sq. m. per hr. The lowest point observed in men was 34.9 kcal. per sq. m. per hr. The average skin temperature for women in the hot zone was higher than for men by 1.5°C. and lower in the cold zone by 1.0°C. The conductance of the peripheral tissues for the women was 20 per cent lower than that of the men in the cold zone experiments, representing 20 per cent more insulation against cold. Sweating was less marked in women and did not begin until the environmental temperatures were higher than was the case for the men. In general, there were slight differences in all aspects of heat regulation, and women had a distinct physiological advantage, but especially in the reduction of heat loss and heat production in the warm zone. Values are given for tissue conductance and these indicate about five millimeters additional superficial tissue in women. There is no evidence that this finding is correlated with position in the menstrual cycle. A number of earlier studies have shown a systematic variation in body temperature during the menstrual cycle in women. Carpenter (72) did not see this in two female gibbons studied for seventeen menstrual cycles. Nonbasal vaginal temperatures were taken at two hour intervals from eight a.m. to four p.m. for fifteen days. Eight a.m. temperatures were taken for 440 days on one animal and for 267 days on the other. Mean vaginal temperature at eight a.m. was found to be 37.5°C. Significant variations were found in diurnal temperature. No significant differences were found in the eight a.m. temperature during normal and menstruating periods or for other phases of the cycle. The study was made in the warm climate of Puerto Rico. Since no seasonal curve was in evidence this study suggests that observations on women should be procured in a tropical environment. Vollman (73) has recently confirmed the menstrual cycle temperature in women. The daily morning rectal temperature in women between two menstrual

periods is a diphasic wave-formed curve with a drop in the postmenstrual phase, a characteristic increase in the intermenstrual period, a larger increase in the premenstrual period followed by a sharp fall shortly before menstruation (284 cycles and thirty-seven women). This is not noted in pregnancy but is resumed again at the first postpartum menstruation. Men, infantile girls, and postclimacteric women do not show cyclic fluctuations of the morning curve. The interval between the intermenstrual pain and the intersecting point of the temperature curve with the temperature average of the cycle seems to define the interval between ovulation and the refunctioning of the corpus luteum.

Many lines of evidence have indicated the relatively small contribution of the resting muscle mass to basal heat production and the corresponding importance of the vital organs. This conclusion is confirmed by Milhorat, Hardy & Fair (74) who studied fifty normal individuals and one hundred patients with muscular disease. Investigations of the metabolism of creatinine and creatine furnished data for computing the total functioning muscular mass of the body and for estimating the reduction in muscular mass due to wasting. In patients who had not reached puberty muscular wasting (up to 60 per cent of the total muscular mass) had no effect on heat production. In adults muscular wasting was often associated with decrease in total heat production but this change was only slight in comparison with the reduction in muscular mass and in the excretion of creatinine. These observations held for all types of muscular disorders except myotonia atrophia in which decrease in heat production was greater than the change in muscular mass and function. Hemingway & Hathaway (75) using a refined action current method for the detection of the onset of shivering have been able to detect a small component of chemical regulation on cold exposure in animals. This increment is of the order of 7 per cent of the basal value and occurs before there is a detectable increase in muscle fiber action. Forbes & Swift (76) have criticized the evaluation of the dynamic effects of nutrients as directly observed at planes of nutrition below that of energy equilibrium. Heat increments based on the heat production of fast are in error by the amount of the dynamic effects of the body nutrients spared. Treichler & Mitchell (77) have examined the related problems in rats exposed to different temperatures at different levels of nutrition. Black & Swift (78) have recently been

unable to confirm earlier evidence both in large animals and in the rat, which indicated that the critical temperature was affected by the plane of nutrition. The published data do not offer at this time any clear resolution of the problem of critical temperature in relation to plane of nutrition as a generality for both large and small animals. Recent results emphasized the hazards of extrapolation from one species to another, particularly when the extrapolation involves the temperature economy and the species concerned differ radically in size and feeding habits. Barnes (79) has discussed the utility of basal temperature in differential diagnosis of thyroid conditions.

Forbes, Dill & Hall (80) have reported on the longer term adjustments in the volumes of blood and tissue fluids on changing from a northerly to a southern climate. On changing to a hot damp climate a small increase in the volumes of both blood and plasma, both absolute and relative to body weight and to surface area, was observed. The average change was plus 5 per cent, but the range was from minus 6 per cent to plus 12 per cent. Interstitial fluid, defined as the fluid outside the cells and outside the blood vessels, decreased in the heat 11 per cent on the average, but the range was from minus 34 per cent to plus 26 per cent. There was no difference between colored sharecroppers, white sharecroppers, and the laboratory workers while in Mississippi in respect to plasma volume per unit of surface area, but interstitial fluid volume was 25 per cent higher in negroes than it was in white sharecroppers or the white laboratory workers. Asmussen (81) reported for the same climatic shift and the same subjects that an increased blood volume and pulse rate compensated effectively for the demands by the hot humid climate on cardiac output. This compensation was not complete in prolonged work.

Remarks

Articles in which the principal emphasis was upon peripheral circulation in a broad sense and those dealing specifically with temperature receptors and sensation have been omitted. Reviews in 1942 on the peripheral circulation by Hertzman (82), on the physiology of the skin by Baird, Lever & Spies (83), and on receptors by Hartline (84) cover a large part of the available literature on these topics. More recent references on the peripheral circulation (85 to 93 inclusive) and on receptors and sensation

(94 to 101) are listed but not discussed. Several pertinent articles in foreign journals were not available (102, 103, 104, 105).

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LIVER AND BILE

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A simple compilation of references to articles concerning liver and bile published during the years 1941 and 1942 would exceed the space assigned to this subject. I have omitted many fields which could be represented only by brief abstracts and these can be found in the various abstracting journals. Many important data undoubtedly have escaped my attention and others have been omitted purposely because the general import of the facts seemed to have been established during the years prior to this review. Attention is directed to the less clearly established fields which are emerging, or seem about to emerge, with new concepts of hepatic physiology. Even with this limitation of the subject, completeness is impossible and the reader is referred to review articles of the individual subjects for completeness of detail.

Hepatic Resistance to Injury

Necrosis, fatty infiltration, and cirrhosis of the liver may develop in animals fed diets deficient in one or more specific types of foodstuffs, and inclusion of the one or more factors in the diet may prevent hepatic injury or restore the previously injured liver. An excess of some of these food factors, as is the case with cystine, also may produce degeneration and cirrhosis. Additional exposure to mildly or severely hepatotoxic agents, such as alcohol or carbon tetrachloride, also has been used to demonstrate the protective value of certain substances as well as the lowered resistance of the liver when certain deficiencies exist. The storage capacity of the injured liver is definitely reduced; glycogen, protein, various vitamins, and other food accessories apparently are able to maintain life, to give some degree of protection to the liver, and to aid in its repair although present in subnormal amounts. There is no evidence available which would indicate the presence or absence of a common factor in the various injuries to the liver produced by disease, hepatotoxic agents, or dietary deficiencies. Likewise, protection of the liver by replacement of dietary deficiencies or additional substances may be limited to the type of experiment

under discussion. There are many contradictory opinions in the field of hepatic resistance and injury but there are few contradictory experiments. The many interesting facts concerning the resistance of the liver accumulated in recent years give rise to the hope that a clearer concept of the detoxicating function of the liver will be developed soon.

Lillie, Daft & Sebrell (1) found that cirrhosis of the liver developed in rats which were fed a diet low in protein and very low in the sulfur-containing amino acids. The pathologic changes were more severe in rats when 20 per cent alcohol was substituted for drinking water, given with the same diet. When a diet low in protein and fat but with added cystine, was fed with alcohol, cirrhosis developed consistently (2). Development of cirrhotic changes was prevented by inclusion of casein, choline, and methionine, singly or in combination with the diet. Lowry *et al.* (3) of the same group of investigators found that, within six days after the addition of choline or casein to the diet of these cirrhotic rats, there was a marked decrease in the amount of fat in the liver cells and there was evidence of hyperplasia of liver cells. With continued treatment, hyperplastic changes increased until repair was complete except for the excess of fibrous tissue, which did not seem to be altered. In another series of experiments (4) in rats fed diets poor in protein and choline, a peculiar cirrhosis developed. This was characterized by fatty infiltration and by the appearance of a hyaline substance in liver cells and various phagocytic cells in the liver and in the spleen and lungs. Necrosis and hemorrhage were noticeably absent. Correction of the major dietary defects caused prompt regression of the fatty changes and increase in the size and number of the hepatic cells, but the fibrous trabeculation and lipoid phagocytes were persistent. In another paper, Daft, Sebrell & Lillie (5) suggested that the experimentally produced cirrhosis and hepatic necrosis and hemorrhage might be separate and distinct entities because choline or methionine had a preventive action on the development of cirrhosis in rats which were fed a diet low in protein, poor in choline, and containing alcohol, while cystine or methionine prevented the occurrence of hemorrhage and necrosis. They did not, however, vary the incidence of the hemorrhage and the incidence of necrosis independently of each other. The work of this group of investigators clearly emphasizes the need for detailed study of hepatic injury from various causes in an attempt to separate different types of hepatic injury

and to determine relationships between cirrhosis, necrosis, hemorrhage, fatty degeneration, lipoid changes, hyaline changes, and regenerative changes of the liver.

The addition of cystine as 10 per cent of the diet fed to young albino rats led to extensive hepatic necrosis and hemorrhage in a few days (6). In the rats that survived the initial acute lesion, fatty infiltration of the hepatic cells and cirrhosis appeared after two or more weeks. Milder changes were noted in rats fed diets containing 5 per cent cystine. Nine different diets were used by Earle & Victor (7), with the addition of 5 and 10 per cent excess dietary cystine. It was evident that the components of the diets influenced the lesions produced by cystine. Cirrhosis was delayed by a diet rich in lard (20 per cent) and cod liver oil (5 per cent) but not by a diet containing butter (25 per cent); fatty infiltration was most marked in association with the low protein, high fat diets; necrosis was most marked with synthetic diets, and hemorrhage was least severe with low fat diets. Choline, added to the diet low in protein and high in fat, inhibited fatty infiltration but did not protect the liver against damage by cystine. There was no apparent correlation between fatty infiltration and the incidence or degree of cirrhosis. Earle & Kendall (8) found that rats fed cystine excreted large amounts of sulfate; the same occurred when methionine was fed. When cysteic acid was fed, only relatively small amounts of sulfate were excreted. Hepatic necrosis and cirrhosis developed in the rats fed cystine or cysteic acid but not in those fed methionine. Smythe (9) showed that slices of rat liver suspended in buffer solutions formed hydrogen sulfide from added cystine or cysteine, with the production of pyruvic acid and ammonia. Miller, Ross & Whipple (10) gave methionine or cystine by mouth or by vein to dogs which had undergone depletion of protein. Twenty-five hours later they exposed the dogs to chloroform and found that the methionine or cystine had given marked protection against hepatic injury. Other nonsulfur-containing amino acids alone or in combination were ineffective. Feeding of beef or administration of plasma protein by vein also were of protective value, perhaps because of the sulfur-containing amino acids included in these substances. It is further suggested that hepatic injury by chloroform is the result of possible alterations of enzyme systems due to the withdrawal or inactivation of sulfhydryl groups by chloroform. Griffith and his associates (11) have shown that the complex of cobalt and cystine formed *in vitro* is relatively

nontoxic and that the toxic action of cobalt or nickel may be reduced by separate administration of cysteine. Methionine and cystine were also effective, and they concluded that poisoning by cobalt or nickel might be due to the fixation and loss of sulfhydryl compounds in tissues and resultant interference with oxidative mechanisms.

Spellberg, Keeton & Ginsberg (12) produced fatty livers and cirrhosis in guinea pigs and rabbits by diets containing 20 per cent fat as butter or cottonseed oil while no changes were produced by equal amounts of hydrogenated fats or by the unsaturated fats fed as only 5 per cent of the diet. Winter (13) studied utilization of fatty acids by rats treated with carbon tetrachloride and fed diets which were fat-free or which contained fats with high or low iodine numbers. He found no significant difference in the loss of fat between normal and treated animals in the group that received fat-free diets. Marked reduction in utilization of fats occurred in the treated animals which received stearate but the difference was less well marked in the animals which received unsaturated fats. Poisoning by carbon tetrachloride does not increase synthesis of fat, but the hepatic damage results in decreased utilization of fatty acids unless large amounts of unsaturated fatty acids are present. Ravdin's group (14) have data on the lipid and glycogen content of the livers of man and of animals in the presence of hepatic damage as influenced by dietary factors. The glycogen content is largely dependent on the amount of carbohydrate or carbohydrate-forming substances available. Adequate protein in the diet is essential to prevention of hepatic injury. Whipple (15) described the lack of resistance of the liver against chloroform when there is a protein deficiency. He also showed that animals deficient in protein were more susceptible to the toxic action of many drugs or to various infections. Jolliffe & Jellinek (16) have written an excellent review of the association of alcoholism and cirrhosis and have included avitaminosis and other possible etiologic factors. Beazell and his associates (17) studied liver function by means of serum bilirubin, urine bilirubin, bromsulfalein clearance, excretion of hippuric acid, serum phosphatase, fasting blood sugar, fructose, and galactose tolerance. Dogs which had received alcohol (3 cc. per kg.) showed no abnormality ten hours later except for some retention of bromsulfalein by four of the nine animals. Results of these tests performed on ten normal human subjects were not abnormal ten hours after the subjects

had consumed a comparable amount of alcohol. Newman and his associates (18) added alcohol to perfused livers of cats and found that alcohol was metabolized directly by the liver but the functional activity of the liver was depressed so that the consumption of oxygen and the concentration of liver glycogen decreased while the blood lactic acid was increased. Ethylene, diethylene, and dipropylene glycols were not utilized but had a similar depressing effect on consumption of oxygen. Propylene glycol decreased the consumption of oxygen by the liver but increased the glycogen content and decreased the utilization of dextrose. Harris, Anderson & Chen (19) found that the alkaloids, senecionine, integerrimine, jacobine, longilobine, and spartioidine, produced necrosis of the liver associated with congestion and hemorrhage. Repeated administration of the drugs produced hypertrophy of the hepatic cells but no instance of cirrhosis was observed. Isatidine, pterophine, and scleeratine (20), and monocrotaline (21) were found to have somewhat similar action on the liver.

Wakim & Mann (22) used the Knisely technique to study the intrahepatic circulation of blood. They found about 75 per cent of the hepatic circulation to be inactive under ordinary conditions; some of the sinusoids were full of motionless blood while others had no blood in their potential lumina. The branches of the hepatic artery supply the hepatic parenchyma with arterial blood by emptying directly into the sinusoids of the lobules, by emptying into the radicals of the portal vein just before the latter empty into the sinusoids, or by anastomotic branches between the hepatic artery and portal vein in the interlobular spaces. Electric stimulation of the vagus nerve had no perceptible effect on the intrahepatic vessels (23). Stimulation of the hepatic plexus caused constriction of the sinusoids and the arteriovenous anastomoses. When stimulation was discontinued, the previously inactive sinusoids became very active and remained so for more than twenty minutes. Inhalation of carbon tetrachloride produced immediate vasoconstriction of the intrahepatic circulation of the rat, but when it was continued for thirty minutes the primary vasoconstriction disappeared and degenerative changes became apparent only in four to eight hours (24). During this period hepatic enlargement with fatty degeneration, hydropic changes, and hemorrhage could be detected in the liver. At the same time the liver became anemic as its vessels became obliterated by encroachment of swollen hepatic cells.

Within two to four weeks after cessation of administration of carbon tetrachloride the liver gradually returned to the state it was in before exposure to carbon tetrachloride. Repeated inhalation of carbon tetrachloride produced cirrhosis, during which important vascular changes could be observed. There were early vasoconstriction during the exposure to carbon tetrachloride and prolonged secondary obliteration of the vascular channels, chiefly the venous ones, produced by encroachment of the swollen degenerating cells of the liver. After the cirrhosis was advanced, accumulation of debris and growth of connective tissue led to further collapse and obliteration of the vessels. Direct injury to the walls of the blood vessels led to numerous small hemorrhages into the substance of the liver. The vascularization of the regenerated regions of hepatic parenchyma was mainly arterial and had no definite pattern. The lobular pattern was completely distorted and the central veins were displaced. The larger arterial and venous channels became very tortuous.

In rats exposed to carbon tetrachloride vapor for thirty minutes three times each week, extensive hepatic damage and hypoprothrombinemia developed and usually the rats died from massive hemorrhage into the gastrointestinal tract (25). With uniform exposure to carbon tetrachloride, adult male rats, fed 20 gm. daily of a diet composed of 8.8 gm. of cracker meal, 8.8 gm. of lean meat, 1.6 gm. of lard, and 0.8 gm. of mixed salts and vitamins, lived from thirty-six to forty-two days. In another group of experiments, half of this diet was replaced with isocaloric values of cracker meal and sucrose, meat, or lard. Considering the duration of life of the rats on the mixed diet and representing the protective value of the mixed diet as 100, the investigators obtained the following protective values: carbohydrate 146, protein 100, and fat 91. In another series (26), three fourths of the mixed diet was replaced with isocaloric values of the above foodstuffs and the following protective values were obtained: carbohydrate 205, protein 104, and fat 59. In sections of the liver taken from other animals which had received the same diets and which received carbon tetrachloride for two to four weeks, the protective value of the diets was evident. The amount of necrosis and hemorrhage was least in the rats that were fed the diet rich in carbohydrate and most in those that were fed fat. The amount of cirrhosis was not greatly different in any of the groups. The regenerative changes, as indicated by the nodules of rapidly growing new hepatic cells, were

most marked in the animals which received additional protein. Post and his associates (27) found that the development of, and the recovery from, carbon tetrachloride cirrhosis of rats was the same whether the amounts of yeast fed were grossly inadequate or were in excess of the requirements for normal growth. The consumption of food was found to modify the severity of the cirrhosis. Rats fed 8 to 11 gm. of the basal diet daily had more severe lesions than those fed 14 gm. daily. The addition of brewers' yeast to the diet had effects similar to the added amount of food.

Haig & Post (28) found that the livers of rats which recovered from cirrhosis caused by carbon tetrachloride contained much less vitamin A than livers of normal rats which received the same diet. Haig & Patek (29) determined the vitamin A and carotinoids of plasma and liver in patients having Laennec's cirrhosis, compared with others having various other pathologic conditions. The vitamin A and carotinoid content of plasma and liver was lower in those with cirrhosis, and the authors expressed the belief that the value for vitamin A in the blood can be taken as a crude index of the severity of the disease. Stewart & Rourke (30) found a rough correlation between vitamin A of the blood and vitamin A of the liver in patients with hepatic disease. Very low values for both were found in the presence of obstructive jaundice unless the patients had been given carotene and bile salts previously. Popper & Brenner (31) found no simple parallelism between the vitamin A content of the liver and of the blood in rats taken at various periods while being fed a diet free from vitamin A. Brenner and associates (32) found that young rats lost their vitamin-A stores from the liver in a week but that the livers of older rats contained some vitamin A after thirty weeks of the depletion diet. Popper & Greenberg (33) reported similar observations and also noted that vitamin A could be replaced in the depleted liver a few hours after its oral administration.

Choline, Methylated Compounds, and the B Vitamins

Knowledge of the utilization of choline, methylated compounds, and B vitamins was being rapidly advanced by the work of several laboratories. The presence or absence of these dietary factors markedly alters growth and metabolism of fat, protein, and carbohydrate. Organs other than the liver are involved, but the striking changes in the liver produced by these substances necessitate

their inclusion in this review. With the aid of these substances in combinations of deficiency and excess, it is possible to produce or cure a biochemical lesion of the liver which can be recognized grossly, microscopically, or chemically by the fat and lipoids deposited in the liver. From the many studies that have been made, it is obvious that the specific nature of the fat and lipoids deposited in the liver may be varied by the procedures involved and that there are several different types of biochemical lesions. The ultimate nature of the physical changes in the liver depends on the nature of the biochemical lesion and the availability of lipoids or the functioning of the lipoid synthesizing mechanisms. Due caution should be used in comparing the results of these studies. Many common factors undoubtedly do exist but any assumption not entirely substantiated by direct experimental data is unwarranted. Reviews of this field have been written by McHenry (34), Best (35), Griffith (36, 37), and du Vigneaud (38).

Longenecker, Gavin & McHenry (39) reported further studies of the fatty liver produced by administration of a beef-liver fraction to rats fed a fat-free diet. Livers of these rats contained large amounts of cholesterol, which was less when lipocaic was given but which was not influenced by choline. While the liver fraction caused a marked increase in cholesterol in the liver, there was no significant change in the ratio of free to ester cholesterol. The fatty livers caused by the liver fraction contained large amounts of acetone-soluble fat which was kept at a normal level by simultaneously feeding lipocaic. The fatty acids synthesized when thiamin, riboflavin, pyridoxin, and choline were given were largely C_{16} and C_{18} acids. Further supplementing the diet with the liver fraction caused a greater increase of C_{18} acids and increased the quantity of unsaturated acids. McHenry & Gavin (40) found that while administration of thiamin alone would cause synthesis of fat from carbohydrate, it would not cause synthesis of fat from protein unless pyridoxin also was present. This vitamin, in combination with thiamin, prevented the loss of fat of rats maintained on a protein diet. The synthesis of fat from protein was evident when pyridoxin, thiamin, riboflavin, and pantothenic acid were supplied. Various combinations of the isolated members of the vitamin B complex did not cause synthesis of fat from protein unless pyridoxin was present. Forbes (41) administered thiamin, riboflavin, pyridoxin, nicotinic acid, and calcium pantothenate

to rats that were fed a fat-free, high carbohydrate diet, containing 10 per cent casein, which produced fatty livers of high cholesterol content. The nicotinic acid caused increased production of cholesterol in the fatty livers. The addition of choline to the diet exerted a lipotrophic action under all conditions studied, but this effect was less marked in the animals which received nicotinic acid. Gavin & McHenry (42) produced acutely fatty livers containing large amounts of cholesterol by feeding crystalline biotin and by administering solutions of biotin prepared from several sources other than liver. The biotin activity was prevented by the simultaneous feeding of egg white. Lipocaic or inositol prevented the increase of fat and cholesterol in the liver caused by biotin while choline, which prevents the development of fatty liver caused by thiamin, was ineffective. The effect of biotin was not obtained unless thiamin, riboflavin, pyridoxin, and pantothenic acid were also furnished and is completely similar to the effects of the liver fraction. The action of inositol in this type of experiment on rats resembles that of lipocaic (43). At the Gibson Island conference on vitamins in July 1942, McHenry called attention to enzymatic roles played by the B vitamins in promoting synthesis of fat. Relations to enzymes have been established for thiamin, riboflavin, and nicotinic acid and the essential role of pyridoxin in protein metabolism also indicates its relationship to an enzyme. Similar relationships for pantothenic acid and biotin may be anticipated. No such direct relationship has been shown for choline or inositol, both of which are probably structural entities in the formation of phospholipids.

The fatty livers and hemorrhagic degeneration produced by dietary deficiency of choline or the labile methyl supply were increased in severity by cystine, cysteine, homocystine, and glutathione while taurine was without effect. This difference was not observed when the choline deficiency was so marked that hemorrhagic degeneration of maximal severity occurred without addition of the sulfur-containing compounds to the diet, but was evident only when the same diets had been supplemented with small amounts of choline. In these studies by Griffith & Mulford (44), it was found that betaine, like methionine, could be substituted for choline and that the effectiveness of betaine corresponded to the utilization of only one of the three methyl groups. Creatine did not contribute to the labile methyl supply. Griffith & Mulford (45)

also found that supplements of thiamin, riboflavin, pyridoxin, calcium pantothenate, ascorbic acid, vitamin K, and vitamin P did not affect the severity of the results of labile methyl deficiency. The amount of food taken altered the effects of labile methyl deficiency. The effects might be prevented by partial restriction of the diet and increased by increased consumption of the deficient diets. In further experiments (46) the same investigators found that small amounts of cystine or methionine improved growth and the efficiency of utilization of food in young rats on an 18 per cent casein diet and that the deposition of liver fat was increased by the addition of cystine (0.1 to 0.2 per cent). Large supplements of cystine had no further effect on the requirement of choline. Du Vigneaud, Chandler & Moyer (47) also found that creatine, creatinine, or sarcosine did not enable homocystine to be used in the diet in lieu of methionine. These compounds did not give up methyl groups for the conversion of homocystine to methionine. Moyer & du Vigneaud (48) found a high degree of specificity among derivatives of choline, various betaines, and other compounds in their ability to act as a methyl-donator in young rats given a diet containing homocystine but lacking in methionine. Of the compounds tested, only choline and simple derivatives such as lecithin and phosphorylcholine, betaine, and dimethylethylhydroxyethyl ammonium chloride were found to be effective. Stetten (49) fed ethanolamine-containing isotopic nitrogen to rats that were receiving diets with and without an adequate supply of available methyl groups. The ratio of isotopic nitrogen found in the ethanolamine and the choline isolated from the phosphatides of the animals indicated that ethanolamine was converted to choline even when the diet was sufficiently poor in methyl groups to cause fatty liver. Patterson & McHenry (50) found that triethylcholine could replace choline in preventing hemorrhage due to deficiency of phospholipid. Treadwell, Groothuis & Eckstein (51) added increasing amounts of cysteine to a diet of 5 per cent casein and 40 per cent lard, supplemented with methionine so that the methionine content was equivalent to that of a diet containing 20 per cent casein. No increase in the lipid content of the liver was observed until the total content of dietary cystine exceeded 1 per cent. The lipotrophic action of methionine, given as the free amino acid, was far superior to diets containing equivalent amounts of methionine in the form of casein. Treadwell and associates (52) found that the glucose tolerance of the rat was markedly decreased

in animals with fatty livers produced by diets high in fat and low in lipotrophic factors. In comparing experiments it was demonstrated that there was no direct relationship between the glucose tolerance and the fat or glycogen content of the liver, and that the glycogen content of the liver could be independent of its lipid content. Samuels, Reinecke & Ball (53) found that hypophysectomized rats had less liver fat and more body fat than controls on similar diets of the same caloric content. György & Goldblatt (54) varied the casein content of diets supplemented with thiamin, riboflavin, pyridoxin, and calcium pantothenate. With diets containing less than 10 per cent casein and without the vitamin supplement, cirrhosis of the liver developed within 150 days. The addition of yeast or choline reduced the incidence of cirrhosis while the addition of cystine could be partly neutralized by the addition of yeast or choline.

The use of labeling agents in the study of phospholipid metabolism was recently reviewed by Chaikoff (55). With Fishler, Taurog & Perlman (56), he found that radioactive phosphate was synthesized into phospholipids in slices of liver suspended in buffer solutions but that the synthesis did not take place in homogenized liver. The rate of turnover of phospholipid in slices of liver was rapid since more than a fourth of the newly formed phospholipid was shown to disappear within six hours. Taurog, Chaikoff & Perlman (57) found that anaerobic conditions or the presence of respiratory inhibitors, cyanide, azide, hydrogen sulfide, or carbon monoxide inhibited the formation of phospholipid in slices of liver or kidney. The mechanism of lipid phosphorylation is associated with an energy-producing mechanism of the cells, probably the cytochrome-cytochrome oxidase system because the presence of strong light abolished the inhibitory effect of carbon monoxide. This work appears to link lipid phosphorylation with an enzyme system and suggests that the lipotrophic effects of the labile methyl compounds may be associated also with enzyme activity. Chalecki Witenberg, Taylor & Hodge (58) found that the increased metabolic rate due to the administration of dinitrophenol decreased the content of liver phospholipids and cholesterol in the mouse. Mirsky and his associates (59), by the administration of insulin, increased the concentration of inorganic and organic acid-soluble phosphorus in the livers of fed and fasted rats. Fasting or injection of epinephrine had little effect.

Montgomery, Entenman, Chaikoff & Nelson (60) produced

fatty livers by excision of the pancreas of dogs subsequently maintained with insulin and by complete ligation of the pancreatic ducts. The development of fatty livers could be prevented by daily administration of large amounts of pancreatic juice. The low levels of blood lipids were also increased to normal levels by the treatment (61, 62). The daily administration of lipocaic from 5.5 gm. of fresh pancreas was sufficient to prevent the development of fatty liver in depancreatized dogs maintained with insulin. Since 35 mg. of choline for each kilogram of body weight of the animal was needed to prevent the development of fatty liver, and the amount of lipocaic necessary contained little choline, it was obvious that the effects of lipocaic were not due to its choline content. Dragstedt and his associates (63) found that the fat could be removed from the liver of depancreatized dogs by administration of lipocaic, without increase in the ketones of the blood. The blood ketones were not increased by deficiency of lipocaic as is the case with deficiency of insulin, nor did lipocaic have any effect on the hyperketonemia of deficiency of insulin. Gibbs & Chaikoff (64, 65) have clearly separated the lipid changes that result from interference with other factors in the pancreas. Marked hyperlipemia was obtained only in dogs fed diets containing raw pancreas and deprived of insulin. The rapid infiltration of fat in the liver which accompanies deprivation of insulin was not influenced by the feeding of raw pancreas.

The Liver and Metabolism of Fat

Most of the problem of metabolism of fat centers around the liver, and there is general agreement that the liver is the chief source of the ketone bodies. Soskin & Levine (66) have summarized the present knowledge of the intermediate metabolism of fats. Ennor (67) found that respiring slices of both normal and fatty livers of guinea pigs oxidized fatty acids; in the latter case the fatty livers were produced with carbon tetrachloride or anterior pituitary extract. In the presence of propionic or valeric acid no ketone bodies were produced, in contrast with the presence of crotonic acid. The liver seemed capable of complete oxidation of these acids which contained an odd number of carbon atoms. Stadie, Zapp & Lukens (68, 69) found that slices of liver from diabetic cats formed new carbohydrate from added lactate. The entire increase could be accounted for by the decrease in substances

known to produce carbohydrate, and no additional carbohydrate was produced from the fatty acids of the diabetic liver. The diabetic livers produced no acetic or similar acids although abundant ketone bodies were produced. Approximately four mols of ketone body were produced by the partial oxidation of one mol of fatty acid. This evidence suggests multiple alternate oxidation instead of successive β-oxidation. MacKay and his associates (70) found that the ketosis of the fasting rat was not influenced by the fat content of the liver nor by agents such as choline, methionine, or cystine. The ketosis was less following a diet high in protein. Barker (71) was unable to correlate the excretion of ketone bodies of depancreatized dogs with increased metabolism due to exercise or with administration of dinitrophenol. The increased oxidation of fat was not accompanied by increased excretion of ketone. In addition to possible variations in the rate of oxidation of the ketone bodies found, Barker expressed the belief that there is an alteration in the stoicheiometric production of ketones from fatty acids. Drury & Wick (72) found a decreased excretion of ketone bodies and a lessened concentration of ketone bodies in the blood during exercise of human subjects who were on a ketogenic diet. With MacKay (73), they found that heavy exercise produced an immediate drop in the value for ketone in the blood of rats, followed by an increased amount during subsequent rest. Crandall (74) used the angiostomy method on fasting dogs to determine that the ratio of β-hydroxybutyric acid to acetoacetic acid and to acetone of the blood is the same as the ratio in which the liver adds these ketones to the blood. Other studies, by Crandall, Ivy & Ehni (75), indicated that the liver did not add ketone bodies to the blood until two to four days of fasting had ensued. After this time the addition of ketone bodies might supply up to 50 per cent of the total caloric requirement of the dog. Administration of glucose was definitely antiketogenic. These findings indicate that there are two alternative pathways for oxidation of fat, one with production of acetone bodies in the liver and an alternative pathway not involving ketogenesis. Crandall (76) found that ketosis of fasting was similar in humans and dogs, except for the slightly earlier and more intense ketosis developed by the fasting human being. Using similar methods, Ivy & Crandall (77) found that the fasting or fat-fed dog retained lactic acid in the liver. The administration of epinephrine on lac-

tates did not cause significant retention of lactic acid by the liver and there was clearly no retention in the nonfasting animal.

Carbohydrate Metabolism

Guest (78) found that he could establish the glycogen content of the livers of rats at any desired level, form 0 to 8 per cent of the weight of the liver by variations of the fat, protein, and carbohydrate content of the diet. When food was withheld for twenty-four hours, the level of glycogen was higher in the protein-fed rats than in fasted rats after feeding of carbohydrate. Nutter (79) found that the liver glycogen was not greatly affected by vigorous exercise of fasting rats, but that about three hours after exercise the glycogen content of the liver increased to approximately twice its fasting level. Newburger & Brown (80) found that ether anesthesia caused a loss of glycogen from the liver of about the same amount whether the rats had received a high carbohydrate or high protein diet, although those which received carbohydrate had higher concentrations of glycogen. Huchtemann (81) found that regulation of the glycogen content of the liver by food protein is independent of the glucose-forming potentialities of the amino acids fed. Koepf and his associates (82) found that the rate of synthesis of carbohydrate by slices of rat liver, from pyruvate and from lactate, was markedly increased by the addition of adrenal cortical hormone. Abelin & Althaus (83) found that the decrease of liver glycogen produced by administration of thyroxine to rats could be partially relieved by the simultaneous administration of adrenal hormone, but this procedure was not effective in previously thyroxinized animals. Corkill & Nelson (84) found that administration of adrenal cortical extract, or progesterone, caused deposition of glycogen in the livers of young rabbits. Testosterone, estradiol, and desoxycorticosterone were inactive. Anterior pituitary extract caused deposition of glycogen in normal animals but produced only deposition of fat in adrenalectomized rabbits. Pilgrim, Axelrod & Elvehjem (85) found decreased oxidation of pyruvate in livers from rats deficient in pantothenic acid and biotin. This is taken as indirect evidence that these vitamins may be components of the enzyme systems concerned with the metabolism of pyruvate. MacKay and his associates (86) fed the isomers of alanine to rats in order to observe the formation of glycogen in the liver. The racemic mixture formed glycogen as fast as

the natural form but the *d*-alanine formed glycogen more slowly because of its slower absorption and limited rate of conversion to glycogen. Hastings and his associates (87) fed rats lactate containing isotopic carbon in the α and in the β position. The liver glycogen formed was about 20 per cent of the lactate fed and contained about 3 per cent of the radioactivity. The carbon dioxide expired during the period accounted for about 10 per cent of the administered radioactivity. In another series (88), isotopic carbon was introduced as sodium bicarbonate during absorption of glucose by otherwise fasting rats. After 2.5 hours, about two thirds of the radioactive carbon had been excreted as carbon dioxide and about 13 per cent was present in the liver glycogen formed. Muscle glycogen contained more radioactive carbon than was found after feeding of lactate. Similar experiments with slices of liver, in a suitable medium in which there was no synthesis of glycogen from lactate, pyruvate, succinate, or fumorate, indicated that only traces of radioactivity were present in the glycogen synthesized. This may indicate that glucose is broken down to a three-carbon compound before resynthesis to glycogen or that carbon introduced at any stage of the equilibria between the various components of degradation of glucose is distributed to all of the involved compounds. The active participation of carbon dioxide in the formation of glycogen is of great interest.

Hormonal Inactivation by the Liver

That the liver plays a role in the detoxication of the steroid hormones is indicated by the observations of Selye (89). The toxic effects and anesthetic action of desoxycorticosterone, progesterone, testosterone, estradiol, and stilbestrol were greatly increased in animals from which the greater parts of the livers had been removed. Burrill & Greene (90) implanted pellets of desoxycorticosterone subcutaneously and intramesenterically in adrenalectomized rats. Those implanted intramesenterically were ineffective while those subcutaneously implanted maintained growth and life of the animal. The limited capacity of the liver to inactivate the hormone was demonstrated by animals which received intramesenteric pellets three times the size necessary for effective subcutaneous implantation, as in this case definite effects of the hormone were produced. Somewhat similar experiments were performed with pellets of testosterone and methyl

testosterone implanted subcutaneously, intramesenterically, and in the spleens of castrated rats (91). When equal amounts were absorbed daily, pellets in the spleen were least effective and the methyl compound was slightly more effective. Both compounds were moderately effective when absorbed from the mesentery and most effective subcutaneously. Biskind and his associates (92, 93) demonstrated that the estrogen-inactivating function of the liver is diminished in animals fed a diet free from vitamin B complex. Small pellets of estrogens implanted in the spleens were ineffective in normally fed animals but became effective when the animals were deficient in vitamin B. MacBryde and associates (94) found fatty changes and degeneration of the livers of dogs given estrogenic doses of estrone, estradiol, or diethylstilbestrol. Freed, Rosenbaum & Soskin (95) were unable to find any hepatic changes in rats or dogs given large amounts of diethylstilbestrol or in patients who had received therapeutically effective doses.

Inhibition of Hepatic Carcinoma

The incidence of the production of carcinoma of the livers of rats fed *p*-dimethylaminoazobenzene has been varied by administration of dietary factors which influence the liver. Sugiura & Rhoads (96) found inhibition of formation of carcinoma to be produced by feeding a rice-bran extract and yeast extracts and almost complete protection by a diet of rice and 15 per cent brewers' yeast. The protection was diminished as the amount of yeast in the diet was decreased. Additions of casein to the diet were ineffective. The early cirrhosis produced by butter yellow was treated successfully by the addition of yeast to the diet but there was no effect on formation of tumor once hyperplasia, cholangioma, or hepatoma had developed (97). Miller and his associates (98) used a variety of diets in the study of protection against formation of hepatoma. Diets deficient in protein and the vitamin B complex were the least protective and the maximal protection was afforded by dietary supplements rich in protein and the vitamin B complex. Nutritionally adequate diets offered partial protection against formation of tumor. Du Vigneaud and his associates (99) found a high degree of protection against formation of tumor in rats maintained on diets containing choline, riboflavin, thiamin, pyridoxin, nicotinic acid, inositol, pantothenic acid, and vitamins A, D, E, and K. The addition of crystalline biotin or biotin con-

centrates to this diet greatly increased the incidence of formation of tumor. Kensler, Young & Rhoads (100) found no diminution of the ability of the liver to produce glucuronic acid or to conjugate borneol in rats that were fed butter yellow to the stage of formation of tumor. Cohen, Hekhuis & Sober (101) found a marked decrease in the transaminase activity of livers from rats fed butter yellow, which was increased to normal values by the feeding of yeast.

Bile

Ackerman, Curl & Crandall (102) carried out roentgenologic studies on dogs with bile fistulas, using meals containing large and small amounts of fat, in order to determine the relationship of bile to gastrointestinal motility. Their data did not support the suggestion that bile salts are important factors in the regulation of the motility of the small intestine. Bile fistula dogs which were given a mixed meal exhibited an increase in the rate of gastric evacuation and of entrance of barium into the large intestine. After a fat meal, gastric evacuation was slower than in normal dogs and administration of bile salts with the fat meal brought the gastric emptying time to within normal limits. Peters (103) used anesthetized dogs and found that absorption of water and chloride from intestinal loops was decreased by the presence of bile salts in the following concentrations: 2 per cent commercial bile salts, 1.5 per cent sodium taurocholate, 0.2 per cent sodium glycocholate, and 0.2 per cent sodium dioxycholate. Higher concentrations produced greater effects, and control experiments with equivalent solutions of sodium acetate, glucose, and sodium bicarbonate did not alter absorption of water or chloride. Smith & Crandall (104) found increases in the iron content of the plasma of bile fistula dogs essentially similar to increases observed in normal dogs. The presence of fat in the diet inhibited the absorption of iron in the absence of bile but not in normal animals. Virtue and his group (105) found that sodium oleate introduced into jejunal loops of dogs was not absorbed to an appreciable extent. The addition of gallbladder bile enhanced absorption but sodium taurocholate or glycocholate were without effect. Winter, Van Dolah & Crandall (106) were unable to produce alimentary hyperlipemia in Eck fistula dogs, presumably because of slow absorption of fat which in turn was due to the small amounts of bile salts

excreted by the liver. Winter & Crandall (107), with the angiostomy technique, were unable to detect any increase in the fat content of blood of the portal vein due to the absorption of fat under conditions in which such an increase would have been demonstrable had 10 per cent of the absorbed fat gone by way of the portal vein in normal dogs.

Ivy (108) has reviewed the applied physiology of bile secretion and bile salt therapy. Berman, Snapp, Ivy & Atkinson (109) found that biliary fistula dogs, which lost all their bile, synthesized about 0.5 gm. of cholic acid in eight hours. When different amounts of bile were returned to the duodenum, a level was reached after about two weeks so that about 4.0 gm. of cholic acid were secreted each eight hours. About 12 per cent of the bile salts are lost on each enterohepatic circuit and the constant secretory level is reached when the synthesis balances the loss of the returned bile. Hyperpyrexia (110) produced a choleretic effect in anesthetized dogs but had no effect on unanesthetized biliary fistula dogs. No significant effect was produced on the output of the various constituents of the bile. Alcohol (111) in doses just short of producing ataxia did not reduce the volume of bile secreted but did produce murky bile-containing sediment similar to the bile obtained in hepatitis. There was a definite and consistent reduction in the cholic acid secreted but the excretion of pigment was not greatly altered. Schnedorf & Orr (112) found that the flow of bile in pentobarbitalized dogs could be decreased by reduction of the oxygen content of the inspired air, somewhat in proportion to the decrease in oxygen content. Molitor & Kuna (113) found that anesthesia produced by inhalation of ethyl ether, vinyl ether, and ethylene produced little effect on the flow of bile. Chloroform and nitrous oxide depressed the flow of bile unless the anesthetic agents were given in added oxygen. Ivy and his associates (114) studied the toxic and choleretic properties of seven different bile salts in the dog. Sodium dehydrocholate was the least toxic and the best choleretic, and sodium taurocholate was the least effective. In this study and in one on various preparations from hog bile (115), they have made some interesting observations on the influence of chemical structure on the toxic and choleretic properties of the bile acids. Choleresis produced by administration of bile or bile acids did not increase the rate of excretion of injected bili-

rubin (116) or hasten the removal of bilirubin from the blood after release of obstruction of the common bile duct (117). Grodins, Osborne, Ivy & Goldman (118) found that true hydrocholeretics, such as sodium dehydrocholate, or the ketocholanic acids and cinchophen increase the flow of blood in the hepatic artery. Other substances, such as the conjugated cholates, do not produce this effect. An increase in arterial flow to the liver is not essential for a choleretic response to bile salts. Cantarow and his group (119) found large amounts of estrogen excreted in the bile of dogs for three days after a single intravenous injection of estrone. Longwell & McKee (120) found the biliary excretion of estrogens after the administration of estrone to vary from 1 to 8 per cent of the amount injected while the urinary excretion was from 6 to 14 per cent. Greenberg & Troescher (121) found that about 5 per cent of injected radioactive calcium or strontium was eliminated in the bile of the first three days in rats with bile fistulas.

Cruz, Hawkins & Whipple (122) destroyed red cells by administering acetylphenylhydrazine to bile fistula dogs. The increased excretion of bile pigment corresponded closely to the amount which could have been formed from the quantity of red cells destroyed, and at the same time new red cells and hemoglobin were produced, presumably from the iron and globin of the destroyed hemoglobin. New pigment radicles appear to be readily formed by the dog. Watson & Schwartz (123) expressed the belief that conversion of bilirubin to stercobilin depends on a chemical activity which is peculiar to the intestinal tract. Reduction of bilirubin in human bile produced mesobilirubinogen, which could be oxidized to urobilin IX a, but no stercobilin was obtained. Schwartz & Watson (124) isolated a dextrorotatory urobilin from infected fistula bile of human beings, in contrast to the levorotatory stercobilin isolated from feces. Schwartz, Sborov & Watson (125) added mesobilirubinogen to bile and to acholic feces; this converted part of it to *d*-urobilin but no stercobilin was formed. They (126) also found that feeding of mesobilirubinogen increased the stercobilin of the feces and that incubation of mesobilirubinogen with normal feces or with bile and acholic feces resulted in the formation of stercobilin. It is believed that mesobilirubinogen is the primary chromogen and that formation of urobilin requires the presence of bile while formation of stercobilin requires bile

and feces. Watson, Pass & Schwartz (127) injected protoporphyrin into dogs with bile renal fistulas. The major fraction of the protoporphyrin did not appear in the urine-bile samples nor was there an increase in the bilirubin or coproporphyrin. Salzburg & Watson (128) found that passing protoporphyrin through the livers of rabbits did not convert it to coproporphyrin but that there was some formation of a deuteroporphyrin. Nesbitt & Snell (129, 130) studied the excretion of coproporphyrin by patients with hepatic disease. The degree of porphyrinuria present and the ratio of urinary to fecal excretion of coproporphyrin were fair indices of the severity of hepatic damage.

Additional aspects of the physiology of the liver and bile are discussed in a recent article by Snyder (131). Josephson (132) has reviewed studies of the chemistry and physiology of the bile acids and their relation to diseases of the liver. Greene (133, 134) has reviewed recent literature of interest in the field of applied physiology of the liver and biliary tract. Butt & Snell (135) have summarized knowledge concerning the relation of the liver to vitamin K.

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Division of Experimental Medicine
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SENSE ORGANS

By Hallowell Davis and S. Howard Bartley

PART I. VISION

By S. Howard Bartley

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The eye and the optic pathway are not only the objects of study by those interested in understanding vision, but are uniquely adapted to be utilized as a means of studying general nervous and muscular functions. In fact the visual mechanism receives such varied study that it is difficult to select satisfactorily from the total volume of work done just what may be primarily suited for such a review as the present one. This is true especially where the clinical and other medical fields overlap the purely scientific type of studies, since there seems to be a closer similarity than in decades past. Cognizance of this on the part of the reviewer accounts for the omission of certain titles that might be expected to be found here.

Dark adaptation, night blindness, etc.—Dark adaptation has of late come in for a large share of measurement, not for its own analysis but as an indicator of other conditions such as avitaminosis. In the course of this work, several investigators have found dark adaptation tests to be poor indicators of vitamin A deficiency. Steffens, Bair & Sheard (1) showed that certain normal adults could tolerate a diet deficient in vitamin A for some time without dark adaptation being affected. The microscopic examination of the skin in one case of normal adaptation indicated the presence of avitaminosis A which responded to supplemental vitamin A. Bodansky, Lewis & Haig (2) found that blood plasma concentration is a more sensitive indicator of vitamin A deficiency than is dark adaptation. Even though this may well be true, Yudkin (3) finds that there is no standard level of vitamin A in the blood. There is a specific level for each individual below which the final rod threshold is affected. Jeans, Blanchard & Satterthwaite (4), on the other hand, believe the inconsistent results of various investigators have a rational basis in the variability of the states of

the subject rather than in the fallibility of the test used. In this connection, it is pertinent to note that Pett & Lipkind (5) have obtained results which suggest a diurnal rhythm in the vitamin A content of the blood. Ignorance of this may have proved a factor in the variability of findings reported.

Animal experiments have often proved more clear cut. Morgan (6), working with rats, found the dark adaptation curve in the avitaminotic individual displaced about two log units above normal though it did not differ in form.

The connection between night blindness and avitaminosis A has also been studied. Rauh (7) reported that night blindness and altered color fields resulted from a period of six to seven months maintenance on a diet free from vitamin A, whereas the visual field for movement remained unaffected. Barondes (8) suggests that one of the various causes of night blindness is a spastic disorder of the retinal arterioles, the alleviation of which may be produced by the administration of vasodilator drugs. Wittkower, Roger & Scott (9) concluded that most cases of night blindness seen in Britain are probably of psychic origin. The cases they studied were soldiers and it is possible that, if their conclusions be true, one of the underlying mechanisms involved is the spastic condition just mentioned. In this connection, Yudkin (3) found that amphetamine and alcohol brought about a transient improvement of dark adaptation without producing any rise in the level of vitamin A in the blood. Harman (10), after testing 700 "normal healthy" persons of all ages by their ability to count small white discs in a very dim illumination, concludes that there is a wide range of ability in persons with otherwise normal vision. They go so far as to say that some individuals are so deficient that they "should stay indoors at night." Another study in this connection is that of Craik & Vernon (11) who studied cone and rod adaptation thresholds and noted wide relative differences between individuals. Some have high cone thresholds and low rod; others just the opposite. Simple tasks performed during dark adaptation were related partially to cone and partly to rod thresholds. Ability to interpret vague sensory data obtained in rod vision was dependent upon a number of purely psychological factors, intelligence being one of them. Trumbull (12) noted differences in the adaptation process at various ages between three and fifteen years.

Several direct studies on the photoreceptor mechanism have

appeared lately. Broda & Goodeve (13) found the velocity of the primary reaction of visual purple changes over a considerable temperature range, whereas the secondary reaction has a considerable temperature coefficient at room temperature and is immeasurably slow at −73°C.

Wald (15) finds a genetically fixed parallelism between salinity of environment and composition in the visual system in the euryhaline fishes. The fish that spawn in fresh water possess a porphyropsin system, while those that spawn in sea water have rhodopsin. The presence of both types of visual systems in some euryhaline fishes satisfies the formal requirement of two-component color vision. Wald (16) in studying the sea lamprey (*Petromyzon marinus*) found it to possess the porphyropsin (vitamin A_2) system, a fact which greatly extends the phylogenetic connection of this system with capacity for freshwater life.

Jahn (17) showed that the distribution of screening pigment in the eye of the *Dytiscus* does not systematically vary during the day and so cannot be responsible for the diurnal variations in the electrical responses found. Dawes (14) believes that the disposition of retinal pigment granules cannot always be explained by migration.

Brightness discrimination.—The use of intermittent stimulation (flashes), otherwise known as the flicker method, continues to be one of the most frequently employed techniques for analyzing brightness discrimination and its underlying mechanisms.

Fisher (18) found that the increases in the interval of alternation from flicker to fusion and fusion to flicker was associated with an increasing difference between the critical fusion frequency (c.f.f.) for a dark as compared with a bright surround. This, he asserts, cannot be explained by neural activity but rather by the functioning of such a factor as the "V-substance" earlier postulated by Cobb and by Ross. This hypothetical substance was previously invoked by Fisher to account for what seemed to be the interaction between two distant portions of the retina, on the assumption that neural interaction is definitely limited spatially.

The diurnal fluctuation in the c.f.f. of the electroretinogram of the *Dytiscus* was found to be considerable (19). The response measured here was, of course, the directly recorded electrical activity rather than sensation of human beings or overt response of lower animals.

Crozier & Wolf (20), continuing their work on visual mechanisms by the flicker technique, have used spectral colors to determine the relation between mean critical flash intensity and flash frequency needed to obtain critical response when the light-time fraction of the flash cycle is manipulated. This, they had already done for white. They found that the three parameters of the probability summations giving the "cone" and "rod" curves are modified independently. Several important general implications follow from their findings. The same authors (21), by several procedures used to manipulate the "rod" and "cone" parts of the typical human duplex curve, freed it from the usual overlapping by the extrapolated "cone" curve. When this was accomplished, the resultant curve possessed the form of a probability integral which the lower component of the original duplex curve did not manifest when combined with the "cone" effect. They (22) also used various compositions of spectral lights on the zebra finch. The curves manifest the same general relationships between flicker excitation and wave length as the human "cone" contours under the same stimulation. They deduced that this species should possess mostly green globules in its retina, and subsequently confirmed this deduction by direct examination.

Intermittent stimulation was also used by other investigators to study various aspects of brightness discrimination. Knox (23) outlined a number of conditions which affect the value of c.f.f. Among these are stimulation in the other sense modalities. Halstead (24), in following up his previous study on the Bartley effect, found that the ocular discomfort produced by subfusional flash rates was abolished by immobilizing the pupillary and ciliary muscles of the eye, demonstrating that the discomfort must have arisen from some aspect of the behavior of the intraocular muscles.

Certain comparisons were made by Bartley (25) between the direct electrical record from the optic nerve and the sensory results obtained from short flashes of light. Complex end-results are produced in both cases. In sensation, the phenomena constituting the later part of the total experience were identified with certain of the traditional after-images. A criterion was found at the physiological level for distinguishing "after-effects" from the primary effects. All events preceding the "off" effect itself, which is generally clearly identifiable, were defined as part of the primary response.

Karwoski (26) confirmed Dittler & Eisenmiers' earlier observations that the Purkinje after-image could be elicited from the rod-free area of the retina, and analyzed out certain influences of the surrounding field upon the hue, shape and visibility of after-images. Taylor (27) studied the changes in the size of after-images induced in total darkness. He found no change with head motion if the fixation point moved with the head, but found that the size did change when the fixation control was absent. Hence he concluded that kinesthetic and tactile components of stimulation are the basis of the phenomena in question.

Vujic (28) studied the after-image disturbance connected with disease of the visual pathway, and showed that, among other symptoms, there may be disturbances in color. Gardner (31) demonstrated that the visibility of the spokes on a rotated wheel could be obtained with the camera as well as with the eye, which results favor a theory of the persistence of the after-effect allowing the eyes to summate a variable fraction of the total pattern.

Grether (29) found minimal separable visual acuity to be a better comparative measure than visual angle acuity for comparing the data obtained by various investigators on birds, rats, monkeys, and chimpanzees. Berger (30) points out distinctions between tests of the resolving power of the eye and the usual visual acuity tests, the former being based on the fact that at threshold intensity the minimum visual separation between two points is independent of their distance from the eye.

Color vision.—Shlaer, Smith & Chase (32) measured the relation between visual acuity and illumination with blue and red light. For the latter, the data represented a continuous curve of cone vision. The blue light data manifested two distinct curves with the abruption at about 0.03 photons, below which pure rod vision was represented. Both the broken C and the grating were used as test objects. Determination of maximum visual acuities showed that for the grating the pupil had to be larger the longer the wave length, so as not to be the limiting factor. Various other findings in addition to these were also reported.

Wald (33) measured the participation of the rods and cones in certain visual functions by measuring the energy ratio of two colored lights when they were equally effective upon cones, and then finding the required energy ratio for the same two lights to be equally effective in stimulating the rods. It was found that

presence or absence of color vision is not a reliable criterion for identifying the activity of cones or rods. Color is still seen when the shift from cone to rod vision is well along though it vanishes before the completion of the shift.

Mandelbaum & Mintz (34) tested the dark adaptation of cones to colored lights and found it to be consistent with Hecht's curves that indicate overlapping sensitivities of color receptors and with the idea of three closely related photosensitive pigments probably developing from a single precursor.

Dunlap & Loken (35) find no clear correlation between color blindness and diet, but assert that repeated large does of vitamin A have enabled certain patients to pass some of the standard color vision tests which they previously had failed.

Illusions.—Sickles (36) utilized a well-known illusion in visual perception (the Ponzo illusion) to test an hypothesis concerning the way the central nervous system functions. His assumption was that nerve impulses arriving in the visual cortex set up areas of potential difference between which electrical interaction occurs in such a way that the consequent electrical vectors produce a cortical image with a structure identical with that in the retina. He believes that the results of his experiments coincide with the calculations from purely physical considerations.

Tussing (37) used reversible visual "illusions" to compare individuals before and after vigorous exercise, and since the reversals differed in the two cases, the procedure was considered a possible index of fatigue.

Visual model and anatomy of the eye.—Bethe (38) set up an oscillator circuit which could be adjusted so that it was quiescent in the dark but active when light fell on the neon tube element of the circuit. With increasing strength of illumination the frequency of discharge increased and the amplitude decreased. During prolonged illumination at constant intensity, frequency decreased. These phenomena correspond to the behavior of optic-nerve discharge with the eye in darkness and in light. The general inferences that always follow from successful models can be drawn.

Johnson & Detwiler (39) report that the abundantly present lipoidal droplets in frogs' eyes are so related to the visual cycle in that they can be identified as retinene. Both retinene and the droplets show a striking likeness in behavior under similar conditions.

Cogan & Kinsey (40) assert that the cornea is bounded on both surfaces by a semipermeable membrane passing water but not sodium chloride. Maintenance of corneal dehydration is required for transparency. Removal of the membranes allows the cornea to swell and become opaque.

Functions of the optic pathway.—Progress in determining what parts of the optic system are critically responsible for the various commonly analyzed visual functions, such as perception of movement, brightness, form, and color, is evidenced by the following findings. The usual attempts to distinguish cortical from subcortical functions have been made.

Halstead, Walker & Bucy (41, 42) report two interesting cases of occipital lobectomy in which the hemianopias were checked most carefully. In one, the macula was spared; in the other, it was not. Macular-splitting held for form, brightness, and color discrimination. This seeming paradox has no solution in any of the present views concerning the human visual system, but represents a sound basis for subsequent thinking and experimentation.

Klüver (43) used the "pulling-in" technique with monkeys following occipital lobectomy, and obtained responses to prolonged as well as to suddenly appearing illumination and to positional differences although the responses were to total flux rather than to brightness. This distinction is an important point in the view he has developed from his extended studies. He also found that the visibility curves of his animals were similar to those for human scotopic vision.

Frey (44) has put forward a view postulating a primary hypothalamic optic center persisting throughout the vertebrate series. It is primitive in function and determines light-dark reactions and is connected with peripheral adaptation. It may be suggested here that support for the plausibility of this connection with adaptation exists in the fact that a number of "adaptation" phenomena are now believed to be neural. This theory may possibly need to draw the line between the neural and photochemical adaptation.

Smith (45) in his extended series of ablation experiments (using guinea pigs in this case) has concluded that visual response to movement is under multiple control of cortical and subcortical centers, in such a way that the cortex is critical in the response to isolated patterns while the subcortical centers are critical in the response to the usual complex striated patterns. That movement

vision of the guinea pig appears to be virtually unchanged by cortical removal is explained by the fact that the guinea pig seldom responds to the movement of isolated objects.

Grether (46) has deduced from the similarity in brightness contrast responses in man and chimpanzee that this function is probably mediated subcortically. This conclusion is based also on the fact that certain perceptual functions such as tendency to perceive "brightness constancy" are stronger in certain monkeys than in man and therefore are not subcortical. It is not clear how this conclusion and the findings of Klüver are to be reconciled, for the latter distinguishes between brightness discrimination and reaction on the basis of total light flux and concludes that brightness discrimination depends on the cortex.

Jahn & Wulff (47) locate certain diurnal variations in visual functions in the nervous system rather than in the retinal pigment, since migration of the pigment does not coincide with the diurnal variation. We have here another function which seemed most probably to be determined photochemically but which has at least temporarily been assigned to neural function.

Akelaites (48) finds no support for the idea that unilateral cerebral dominance exists in the elaboration of visual perception.

Kravkov (49) from the instillation of epinephrine and pilocarpine into the eye has found that color vision is affected by these sympathico- and parasympathicomimetic drugs. From this he concludes that the dependence of color perception on the autonomic nervous system might account for its seasonal fluctuation described in recent papers.

Recent papers also include direct electrophysiological studies of the visual pathway. Adrian (50) has noted definite differences in the cortical responses of cats and monkeys to illumination of the eye. The feline response tends to be short and terminate abruptly whereas the activity in the monkey cortex may persist for ten seconds or more beyond the duration of the flash. This difference he also finds in the retinal responses of the two animals. Bartley & Bishop (51, 52) have recorded the optic nerve discharge in the rabbit and the cat. In these two species they find certain overall differences in the envelope of the responses although there is more of an underlying similarity than is superficially apparent. The response of the cat is not as detailed in appearance as that of the rabbit, and therefore the latter is more easily analyzed. The results

indicate that the differences may be due to the timing of the firing of the individual components involved. This same factor makes for differences in the cortical responses recorded, and the caution is injected here that in complexly organized responses a smoothing-out of the recording line does not always indicate cessation of activity. They found that shifts in flash duration and intensity systematically alter the discharge picture; these facts leading to some fundamental deductions.

Crescitelli (53) identified certain types of oscillation in the insect eye associated with various degrees of dark adaptation. Both butterflies and moths were used. He had considerable success in obtaining details of activity, and several different types of response were identified.

Bernhard (54), in studying the electroretinogram of the tortoise (*Testudo graeca*) which possesses a practically pure cone eye, found that the record does not change shape with alterations in dark adaptation, and no positive PI component has been observed.

Stanton (55) applied Householder's neural mechanism theory to monocular depth perception, utilizing the calculated change in shape of the lens and the resulting tension of the ciliary body as functions of object distance. The Weber ratio varied as a small positive power of the distance for nearby objects, while for distant ones monocular discrimination was impossible.

Drugs.—In reviewing the work done with drugs, the question arises of whether the pharmacological studies on eye muscles pertain more significantly to eludicating visual function or to neuromuscular activities in general. We need mention only the studies of Luco & Salvestrini (56), Armstrong (57), Heath & Sachs (58), Sachs (59), Sachs & Heath (60, 61, 62), and Sachs & Yonkman (63). All of these studies appear to be very well conceived and thus very successful in promoting the understanding of neuromuscular function. The concepts set forth in the paper of Sachs & Heath (60) are especially clear and basic for studies on sensitization by drugs.

Dichburn & Steele (64) assert that doses of potassium bromide up to 25 grains produced no detectable change in dark adaptation. Neither did such doses increase the variability of observations. An average user of tea and coffee showed a small but definite increase in adaptation rate when given 10 grains of caffeine, and an appreciable effect with 6 grains.

Reviews.—In addition to the individual studies on vision, several other publications which are important to students in this field have appeared. Walls (65) has produced a very able and exhaustive work on the phylogeny of the vertebrate eye. Volume VII of Biological Symposia, edited by Heinrich Klüver (66), contains the papers on visual mechanisms read at the Fiftieth Anniversary Celebration of the University of Chicago, including an additional four other papers. The contributors were Hecht, Krause, Wald, Gellhorn, Bartley, Case, Marshall & Talbot, Bonin, Garol & McCulloch, Polyak, Walls, Klüver, and Lashley. Polyak (67) also has published a monumental work on the retina. Bartley (68) produced a handbook on vision correlating its experimental sensory aspects with the known neurophysiology underlying them. Fernberger (69) published a review in the *Psychological Bulletin* of basic papers on perception including those on vision.

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SENSE ORGANS

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PART II. SPECIAL SENSES OTHER THAN VISION

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This section reviews the literature of the past year dealing with sense organs and sensory systems exclusive of the eye. If the material seems scanty we must recall that the available literature is almost exclusively American, and furthermore the results of many researches in this field are undoubtedly being withheld from publication because of their military significance. We may only hope that circumstances permit their publication in the not too distant future.

One book that appeared in 1942 deserves special mention in a review of sensory physiology although the book does not report new experimental data or theoretical formulations. It is Boring's *Sensation and Perception in the History of Experimental Psychology* (1). No introduction to a survey of current experimental data could be more appropriate than Boring's excellent summarizing accounts of early experimental work and the development of successive theories and points of view. The scattered current contributions that we will review take on new meaning when viewed against the background so well-pictured in this book.

Hearing

The mechanics of the middle ear have been investigated by von Békésy (2) by means of a probe whose end surface consisted of a condenser about 1 mm. square. From the change in capacity, the amplitude of vibration of a nearby surface could be determined. Von Békésy finds that the transfer of pressure from tympanic membrane to the footplate of the stapes is independent of frequency up to 2400 cycles and represents about a twentyfold increase in force. Separation of the suspensory ligaments of the ossicles causes almost no change in transfer of pressure for frequencies in the middle of the tonal range. It follows that the axis of

rotation of the ossicles practically coincides with their center of inertia. The conical form of the tympanic membrane apparently serves to insure rigid coupling between the membrane, which moves as a whole, and the handle of the malleus. The membrane is freely movable at its edge where there is a well-developed wrinkle or fold of great elasticity. The surface turns on an axis which lies along the edge of the drum membrane, and the amplitude of vibration is greatest beneath the handle of the malleus near the edge of the membrane. Above 2400 cycles there is a change in the mode of vibration of the membrane, which apparently loses its rigidity, and the handle of the malleus lags behind the vibrating part of the membrane in its movements.

Kobrak (3) has rendered the movement of the ossicles of fresh cadavers visible and optically enlarged by means of motion pictures. Taking the pictures in "slow motion" with stroboscopic illumination (4) renders possible analysis of the ossicular movements and emphasizes their complexity. For moderate intensities, an increase of the intensity of a tone is followed by a corresponding increase of amplitude of ossicular movement, but a change of the axis of ossicular vibration is observed at higher intensities.

The action of the pellet-type artificial drum, which when placed in contact with the stapes under slight pressure results in an improvement of hearing, has been analyzed by Lowy (5) on the basis of animal experiments employing the aural microphonics (cochlear response) as an indicator. Lowy believes the improvement is due to an increase in the stiffness of the vibrating system and to a screening effect. The same author (6) has also shown that interference with the sound-conducting mechanism, such as constant pressure on the malleus, causes a gradual change in phase of the aural microphonic similar to that caused in a simple vibrating system by increased stiffness. There is no abrupt change of phase or complete disappearance of response during the phase-shift. Lowy points out that this observation meets certain objections recently repeated by Pohlman (7) to current interpretations of cochlear mechanics, with especial reference to hearing-loss in otosclerosis. By simultaneous stimulation with air-borne and bone-conducted sound, however, cancellation of the aural microphonic may be produced (8). The result is taken as additional evidence that air-borne and bone-conducted sound both involve the same physical process within the cochlea.

Increased or diminished atmospheric pressure in the middle ear is known to interfere with sound conduction. Wever, Bray & Lawrence (9) have shown by a study of aural microphonics that the effect is produced primarily at the ear drum. Another curious instance of change in transmission attributed to the drum is described by Bray & Thurlow (10), who find that transmission across the ear of the pigeon may be reduced by from 8 to as much as 40 db. when the mouth is wide open. The effect of opening the mouth is apparently to increase the tension on the ear drum. Tension applied through a thread attached to the stapedius tendon in the cat causes a marked reduction in transmission for low tones, but a smaller reduction for high tones (11). In general the stapedius, like the tensor tympani, operates so as to protect the inner ear against overstimulation. Incidentally it causes a significant alteration of the pattern of distortion. In the pigeon the interference between two simultaneous tones and the distortion in the cochlear responses are similar to the same effects in mammals (12). Since the pigeon has a single bone, the columella, in place of the ossicular chain of mammals, the result supports the conclusion that interference and distortion are relatively independent of the middle ear and probably arise in the hair cells of the spiral organ.

The nature of the aural microphonics generated after death has been investigated by Wever, Bray & Lawrence (13) in guinea pigs and cats. Promptly after pithing of the medulla, clamping the heart, clamping the trachea, and poisoning with curare, the aural microphonics are impaired, but a partial rebound immediately after the initial drop is frequently seen. The microphonics then decline relatively slowly and are still present after five hours, but not after twenty-four hours. No simple relation was observed between changes in the microphonics and changes of blood pressure, and the initial fall seems unrelated to body temperature. Apparently in death, the cellular changes are simple, and there is no alteration in the effective pattern of activation. The potentials apparently arise through direct transduction of stimulus energy and not through a liberation of stored energy.

The aural microphonic in man has been studied by Perlman & Case (14) who find that it can be obtained regularly when there is a defect in drum membrane that allows placement of an electrode in or near the round window niche, provided the individual has a normal or nearly normal auditory threshold curve. The poten-

tials often amount to only a few microvolts. As in animals, the potentials reach a maximum, above which they do not increase with further increase in the intensity of the stimulating sound. The authors point out that one should think of the aural microphonic not as synonymous with the physiologic reaction of the spiral organ or with auditory sensation but rather as parallel to them.

Walzl & Bordley (15) produced very small localized lesions of the cat's spiral organ by drilling carefully through the temporal bone over a selected part of the spiral ligament. They were able to refine their technique sufficiently to loosen the basilar membrane and crush or dislodge the spiral organ without tearing either Reissner's or the basilar membrane, or allowing escape of endolymph or causing hemorrhage. Measurements of the threshold of the aural microphonics correlated with subsequent histologic examinations showed that near threshold the response to any frequency is localized to a small part of the spiral organ, but that with increase in intensity there is spread of the response to adjacent areas. The spread is greater for low than for high tones. The regions of optimal response for successive octaves were found spaced at equal distances along the spiral organ. This last proposition is slightly at variance with the earlier "maps" of the spiral organ (16, 17), which showed that the lower octaves were relatively compressed toward the helicotrema. Culler (18) has repeated his earlier experiments and reports that his recent studies of the loci of points of maximal potential associated with a given frequency of stimulation agree satisfactorily with the positions that he found earlier (17) by a similar method. Walzl & Bordley (15) interpret their recent findings as supporting the theory that the aural microphonics originate in the hair cells of the spiral organ.

Galambos (19) has presented further details of his studies of the cochlear potentials elicited from bats by supersonic sounds. The magnitude of the maximal potential generated at each frequency is approximately constant, at a level of a few microvolts, between 10 and 60 kc. Below 10 kc. the voltages are relatively large. The phenomenon of hysteresis was observed for the responses to supersonic sounds corresponding to the effect already familiar in other animals at lower frequencies. Immediately following exposure to maximal or supramaximal intensity, the aural microphonic in response to a less intensive tone is not so great as it

was before exposure to the high intensity, but after a minute or two the microphonic recovers to approximately its original level. Galambos explains the hysteresis effect in terms of the reflex contraction of the intraaural muscles and supports his interpretation by the observation that curarization and also deep anesthesia increase the magnitude of the potential elicited by a steady tone and abolish the hysteresis effect. The generation of potentials in response to supersonic tones, and the evidence for activation of the intraaural muscle reflex by loud tones are taken to indicate that bats hear tones of frequencies up to at least 55 kc.

The comparative auditory sensitivity of man and dog was studied by Lipman & Grassi (20) using eighteen men and eleven dogs as subjects. The canine thresholds were determined by the method of conditioned reflexes for seven octaves from 125 to 8000 cycles. At 125 and 250 cycles the human and canine thresholds were the same, but the human threshold was higher at all other frequencies. The difference increased with increasing pitch. The maximal human sensitivity was found at 1000 and 2000 cycles, but for dogs it was near 4000 cycles. The authors call attention to the fact that the dogs were far more consistent in their responses than were the human subjects!

Auditory fatigue and trauma.—Frequent exposure of human ears to very intense sounds, either prolonged as in high-powered airplanes, tanks, and engine rooms, or brief as in the case of gunfire, has stimulated interest in the effects of such exposure on auditory function. The incidence of partial high-tone deafness, and particularly of a hearing-loss centering at or about 4000 cycles, in individuals who have long been exposed to such hazards has been pointed out by many workers. The observations and conclusions of Rosenblith (21), who studied industrial deafness following exposure to machine noise, of Bunch (22) and of Campbell (23), who have studies the hearing of aviators, and of Shilling & Everley (24), who have measured auditory acuity among submarine personnel, are strikingly similar. Such surveys of auditory acuity do not prove that the loss of sensitivity for high tones is the necessary result of prolonged or habitual exposure to loud noises, but the conclusion seems inescapable that such exposure is at least a contributing cause. Chamberlain (25) determined the auditory acuity of boilermakers before and immediately after exposure to the noise of their daily routine. In addition to finding the high-tone loss in

many of the workers, he observes that the "aural fatigue" may be great and recovery rapid, but that complete recovery does not always take place within fifteen hours of the termination of exposure. He implies that these residual temporary losses may become cumulative when he suggests that adequate intervals of rest between periods of exposure might prevent or delay the onset of permanent cochlear damage. He also points out the individual differences in susceptibility from one worker to another. It is a curious fact that the hearing-loss seems frequently to center at about 4000 cycles. Wever (26) investigated the structural conditions underlying this "tonal dip" by examination of serial sections of a number of temporal bones of persons whose audiograms had indicated a marked local depression of sensitivity. A rigid criterion of selection, which excluded general losses of sensitivity, gave a great preponderance of dips among males, and the dips in nearly all cases were at 4096 cycles. The results of microscopic examination showed a somewhat higher incidence of lesions of inner ear structures in the ears in which large dips were present, but no region was identified as specifically responsible for the local dip. Wever offers the suggestion that the lower basal portion of the spiral organ is one of particular fragility.

"Acoustic trauma" (a transient depression of auditory sensitivity) was produced by Perlman (27) by exposing subjects to intense "pure" tones for a period of an hour. There is some doubt as to the exact intensities employed, as measurements with a noise-level meter gave values ranging from 90 to 118 db. (above 10^{-16} watts per sq. cm.), but the statement is also made that the intensity was "close to the threshold of pain." The order of magnitude of the intensity is obvious, however. A definite relation was found between the frequency of the exposure tone and the resulting hearing-loss, but the greatest loss of sensitivity was not, as one would expect, at the frequency of the exposure tone. The maximum loss of sensitivity was usually an octave above that of the exposure tone. A threshold curve commonly found in early acoustic trauma (with a localized dip at 4096) was obtained in subjects who had been exposed to a tone of 2048 cycles. Sounds of low frequency produced less acoustic trauma than those of higher frequency but of approximately equal intensity. Tinnitus of variable duration followed exposure, and its frequency was not regularly identified with that of the exposure tone but was often identified with the

octave above it. The curious discrepancy between the exposure frequency and the frequency at which function is most impaired is without explanation. The temporary hearing-losses produced in this experiment are transient but seem to be the temporary prototypes of the permanent tonal dips reported by Rosenblith (21), Bunch (22), Campbell (23), and Shilling & Everley (24) in individuals habitually exposed to loud noises.

Kobrak, Lindsay & Perlman (28) also studied hearing sensations and acoustic reflexes under the influence of a fatiguing tone. They divide auditory fatigue into three types: central fatigue; an elevation of the auditory threshold, which is produced either in the cochlea or in the primary acoustic nuclei; and a slowing of relaxation of the intraaural muscles. The latter effect they term "auditory adaptation." The authors conclude that the acoustic stimuli of daily life do not affect the acoustic reflex of the muscles of the inner ear.

Response of single auditory nerve fibers.—A preliminary description was presented by Galambos & Davis (29) of the activity of single auditory nerve fibers in cats. By the use of microelectrodes of the pipette type with openings of not more than five micra in diameter it proved possible to isolate the nerve impulses generated by a single nerve fiber when the ear of the animal was stimulated by tones or clicks. Each auditory nerve fiber responds to only a narrow band of sound frequencies when the sound intensity is sufficient to excite it at all. The frequency-band capable of exciting a given fiber increases as the intensity level is raised. At levels about 100 db. above threshold, tones as far away as three octaves below or half an octave above may be adequate. The fibers of the auditory nerve exhibit no unusually brief refractory phenomena. An increase in the intensity of a tone (of constant frequency) causes an increase in the rate of discharge of nerve impulses in the single fiber. Most fibers reach a maximum of 400 impulses per second at an intensity level about 30 db. above their thresholds. A fiber responds to a continuous tone of constant intensity by a train of impulses of initially high frequency but gradually declining in rate. In all respects the behavior of the individual fibers of the auditory nerve corresponds to that established for other classes of sensory nerves. Impulses in different nerve fibers tend to be synchronized with one another because the impulses arise in definite phase relation with the stimulating sound wave. The results are

interpreted as supporting a "place theory" of hearing, according to which pitch is a function of "where," while intensity is a function both of "how much" of the basilar membrane is disturbed and of an increase in the rate of discharge of many of the activated fibers.

Central Auditory Mechanisms

At the risk of overlap with material covered in other chapters, we may touch briefly on a series of papers dealing with the auditory functions of the central nervous system. Some of them throw light on the functions of the sense organ as well as on those of the centers.

Audiogenic seizures.—The peculiar behavior of rats, involving frenzied running, epileptiform seizures, and sometimes coma, originally called "neurotic behavior" by Maier, has now been shown to be determined primarily by auditory stimulation and is more aptly termed "audiogenic seizure." Two papers deal specifically with auditory aspects of the problem. Morgan & Gould (30) show that the behavior is dependent upon the intensity and frequency of auditory stimulation. The frequencies most effective in producing the seizures, when intensity is controlled, are supersonic for the human ear and lie above 21 kc. Morgan & Galambos (31) showed that with sufficient intensity audiogenic seizures could be produced by tones well within the human audible range, even as low as 500 cycles. Comparison of the intensity necessary to produce seizures with the sensitivity curve of the rat's ear indicates that the intensity above the rat's threshold, presumably corresponding to the loudness as heard by the rat, is the chief factor determining the appearance of the seizure. If the seizure occurs at all its latency is approximately constant at about forty-five seconds, regardless of the frequency or intensity of the tone employed to induce the seizure.

Localization in the central nervous system.—The acoustic area of the cerebral cortex of the monkey has been mapped by Ades & Felder (32) by the method of recording the electrical response of the cortex to click stimulation. The responsive area is the portion of the superior face of the temporal gyrus included in the angle between the posterior and medial borders of the gyrus and is quite consistent from one animal to another. Licklider & Kryter (33) obtained frequency localization within this area in the mon-

key. Eight representative frequencies from 125 to 8000 cycles activated the region differentially. The lower frequencies produce their maximal effects in the anterolateral part of the region, the higher frequencies in the posteromedial. The frequencies are disposed in a sharper and more nearly linear gradient than was found in the cat, and the authors suggest that spatial differentiation in the acoustic system is more fully developed in the primates than in lower forms. Evidence for frequency localization in the auditory cortex of the cat is presented, however, by Walzl & Woolsey (34). Localized lesions in the cochlea of the cat which caused loss of response to a limited part of the frequency range as measured by cochlear potentials (cf. 15) result in the disappearance of cortical potentials to click stimulation in specific parts of the cortical auditory area. With lesions in the basal turn of the cochlea sufficient to cause complete loss of aural microphonics for high tones, there was disappearance of cortical potentials from the anterior portion of the auditory area. Lesions in the apical turn of the cochlea caused a disappearance of cortical potentials from the posterior portion. This projection of the cochlea to the cerebral cortex agrees with the projection previously described on the basis of electrical stimulation of nerve fibers in local regions of the osseous spiral lamina (35).

Stowell & Snider (36) present evidence of a representation of auditory sensibility in the cerebellum of the cat as well as in the cortex. Surface-positive electrical potentials in response to click stimuli appear with maximum intensity in the most medial portion of the ipsilateral half of the lobulus simplex, and small responses are found contralaterally in the lobulus simplex and bilaterally in the anterior portion of the declive and the most caudal portion of the culmen. Responses of somewhat different character also appear from the most rostral portion of the anterior lobe.

Binaural interaction.—Thurlow (37) reports that when, at high intensity, a tone of equal frequency and intensity is added to the other ear there is a very definite change in pitch. The effect is also produced by a second tone of different pitch provided the intensity is high. The pitch shift, as a function of the affected tone, is generally similar to that caused by increase of intensity. The author concludes that his observations demonstrate that a change of subjective pitch occurs in the absence of a shift of the position of maximal stimulation on the basilar membrane, and he therefore infers that

the central interaction responsible for the subjective pitch change is not of spatial character.

Theories of cochlear action.—Macnaughton-Jones (38) has published a further elaboration of his theory of cochlear resonance. No new experimental data are presented, and the adequacy of the physical and mathematical analysis (and some of the conclusions drawn therefrom) may be open to question, but the theory is in accord with the general trend of current thought in that the mass and momentum of the fluid contents of the cochlea as well as the properties of the basilar membrane are considered as the determinants of resonance. No appeal is made to variation of tension of the membrane from one region to another and the cochlea is treated, not as a series of resonators, but as a "single resonator of infinite gradation."

Smell

Hirose (39) undertook to determine whether the sense of smell could be excited by direct application of electrical stimuli to the nasal mucosa. On faradic stimulation he obtained no particular sensation of smell but only one of pain. With constant current he elicited a burnt or smoky odor, but finally concluded that the electrical current itself did not constitute the ultimate stimulus but instead that the smell was due to the electrolytic products of the current.

Cutaneous Senses

Considerable interest has centered about study of the pressure and vibration sensitivity of the skin and the question as to what may be the receptor organs involved. Weddell (40) carried out an anatomical study of the skin of a finger removed at operation, which had previously been tested for tactile sensitivity. He found that in an area of acute tactile sensibility each "spot" is commonly innervated by two or three nerve fibers approaching from different directions and ending in separate Meissner's corpuscles. He suggests that such an anatomical arrangement is the basis for the great tactile acuity possessed by the fingers for it provides for a spatial summation which allows for the recognition of graded stimuli. Gilmer (41, 42), however, decided from a review of the literature that the attempts that have been made to establish correlations between the various cutaneous sensory qualities and their possible receptors have, on the whole, been incon-

clusive. He then studied histologically bits of excised skin that included spots possessed of special sensitivity to touch, pressure, vibration, or temperature and also from regions that were relatively insensitive to pressure and vibration. Anatomical findings of these biopsies are reported in some detail and he is unable to confirm the suggestion that either Meissner's corpuscles or the corpuscles of Pacini function in the sensation of pressure and vibration. Instead he finds a correlation between such sensitivity and the presence of arteriovenous anastomoses of the neurovascular system of the skin that he terms "cutaneous glomus bodies." He gives a detailed description of the histological structure of these bodies and points out the part that they have been shown to play in the function of temperature regulation. From the correlation between cutaneous sensitivity for pressure and vibration with the presence of the glomus bodies, he suggests that in addition to its function in the regulation of temperature the glomus body serves as a receptor, if not the receptor, of cutaneous pressure and vibration. In support of this suggestion he points out that the glomus is provided with a richer afferent arterial nerve supply than the sections taken as controls in which the tissue was less sensitive. In another paper Gilmer (43) finds a relationship between sensitivity to cold and the presence of sweat ducts, in that 19.1 per cent of 2,784 sweat duct openings are highly sensitive to cold stimulation while only 10.7 per cent of 2,784 ductless spots were cold sensitive. The difference is stated to be statistically reliable. The author explains the correlation in terms of temperature conduction in the skin and in terms of neurovascular activity.

Nafe & Wagoner (44, 45) present additional data on the subject of sensory adaptation, with particular reference to pressure adaptation, and conclude that adequate pressure stimulation consists only of movement due to the adjustment of tissues in which end-organs are imbedded. The adjustment requires time and the authors' data indicate that the time of stimulation and "adaptation time" coincide, in this case at any rate. This they interpret as showing that adaptation is due to loss of effectiveness of the stimulus rather than to any loss of sensitivity on the part of the end-organ.

Yacorzynski & Brown (46) studied the variability of the vibratory threshold as a function of amplitude and frequency of mechanical vibration. Their particular concern was to find out how

many determinations of vibratory threshold were necessary for adequate reliability. Large fluctuations in the thresholds of the subjects on different days led them to the conclusion that it was necessary to study an individual case over a number of days in order to get a good approximation of his actual threshold under their conditions of experimentation.

Vibratory sensitivity, like pressure sensitivity, is shown by Weitz (47) to be a function of skin temperature. As the skin temperature is increased, vibratory thresholds decrease to a minimum, and with further heating the thresholds rise again. With decreasing skin temperature the vibratory threshold shows a continuous rise. These effects are shown to be not due to changes in skin conductivity nor to a gross vascular action. From the similarity of the relation to temperature of the thresholds for vibration and for pressure, which includes the close approximation of the values of the temperature at which sensitivity is a maximum, Weitz concludes that the functions are similar and that the mechanisms mediating these two sensory experiences are intimately associated if not identical. In a later study (48) he finds a similar function for the threshold of pain produced by an electric shock, and suggests that a chemical theory is necessary to explain the relation to temperature shown by the sensations of vibration, pressure, and pain. It may be pointed out that, however, in regard to pain no evidence is presented against direct electrical stimulation of nerve fibers rather than the activation of special sensory endings that Weitz apparently assumes.

The temperature sensations of warmth and heat have been investigated by Herget, Granath & Hardy (49, 50, 51). Radiation is the method employed for warming the skin and has the advantage of allowing accurate control of intensity and area. It also eliminates the arousal of unwanted sensations such as touch. By means of a half-sector disc and appropriate shutters, either steady or intermittent stimulation could be employed, and by means of the interrupted stimulation, a fusion frequency corresponding to the critical flicker frequency in vision could be determined. The fusion frequency for the sensation of warmth or heat increases with the intensity of the stimulus, rising rapidly at low intensities and less rapidly when the radiation intensity exceeded 10^{-2} gm. cal. per sq. cm. per sec. Estimates of the intensity of the subjective sensation as a fraction of the sensation evoked by a standard intensity

were also made, and it was found that the strength of the sensation so judged rose with intensity. The form of the function closely resembles that for the fusion frequency. A linear relation of both functions, when plotted on a logarithmic intensity scale, for intensities below 10^{-2} gm. cal. per sq. cm. per sec. indicates adherence to the Weber-Fechner law for this limited range of intensities. Direct studies of thermal discrimination made by successively exposing neighboring regions of the skin of the forehead to radiation of slightly differing intensities show that the Weber increment may either change with the intensity or be a constant, depending on the range. From the behavior of the increment it is inferred that three types of receptors are stimulated—first, a warmth receptor; second, a heat receptor, which gives rise to a sensation that differs subjectively, as well as in the behavior of the Weber increment, from mere warmth; and, finally, pain receptors. The entry of the sensation of heat in addition to warmth occurs at an intensity of radiation of about 10^{-2} gm. cal. per sq. cm. per sec., while the pain threshold has previously been reported (52) as 24×10^{-2} gm. cal. per sq. cm. per sec. Herget, Granath & Hardy infer from the trend of the curves relating least perceptible increment to the intensity of stimulation that some pain neurons are excited by as little as 18.5×10^{-2} gm. cal. per sq. cm. per sec. and that excitation of the "heat" neurons probably begins about 5×10^{-2} gm. cal. per sq. cm. per sec. lower than that at which the sensation is first recognized.

The same authors (50) report two types of spatial summation for the sensation of warmth. One type occurs peripherally over an area of 3 to 5 sq. cm. (of the forehead). For areas less than 3 sq. cm. the areal discrimination of warmth is relatively poor. Discrimination is better and practically constant for areas larger than 3 sq. cm. The authors believe that this type of peripheral summation probably occurs between the branches of a single nerve fiber. The other type of spatial summation occurs centrally between different fibers, as from the two hands. Study of the spatial summation of heat (as opposed to warmth) shows that there is spatial summation for this sense also, but to a lesser degree than for either warmth or cold (51). The findings are interpreted as additional evidence that heat sensation is mediated by its own receptor type and is not a "synthetic sensation."

The influence of the sympathetic nervous system on sensitivity

to heat and cold and to certain types of pain associated with exposure to severe cold is reported by Hyndman & Wolkin (53). A cold object feels warmer and a hot object feels cooler to the hand from which the sympathetic nervous supply has been eliminated than to its normal mate. Sympathectomy diminishes and almost abolishes the aching and stinging pain that results from exposure to severe cold, but in the sympathectomized zone of the skin (when tested alone) there seems to be no alteration in the sensibility to touch, pain, and temperature. Evidence is presented to show that the results are not due to a mere change in the cutaneous temperature occasioned by sympathectomy. While no attempt was made to prove whether the altered interpretation of temperature and the diminution in the pain elicited by severe cold are dependent on the interruption of "sympathetic afferent" or "sympathetic efferent" fibers, two cases are described in which the lower extremities were supplied by sympathetic fibers but were devoid of somatic innervation and in which certain types of sensation could be evoked by certain stimuli.

Andrews & Workman (54) describe an application of the method of radiation to a study of the pain threshold in dogs. The animals respond by characteristic twitching or jerking of the stimulated skin when the pain threshold is reached. It is shown that drugs affect these thresholds in much the same way as in man, and the observations seem to open the way to further study of the pain sensation in animals.

Study of the central representation of cutaneous tactile sensibility in the cerebral cortex of the monkey has been reported by Woolsey, Marshall & Bard (55). A central "mapping" of the cutaneous surface by the method of cortical electrical potentials had previously been reported by these same authors, but further details of the "map" are now available.

The Mechanism of Sense Organ Function

Bernhard, Granit & Skoglund (56) report the study of the process of accommodation in the nerve trunk with a view to finding out to what extent a nerve discharging repetitively in response to slowly rising electrical stimuli can serve as a model sense organ. The best defined end-point of response is obtained from microelectrodes inserted into the muscle innervated by the nerve chosen for stimulation. The authors show that for slowly rising stimuli a

"breakdown of accommodation" may take place and the nerve fibers then fire repetitively. The authors believe that a nerve fiber in this condition may serve as a model sense organ, and they assume that a slow "generator potential" is the mechanism which fires the axon repetitively in sensory end-organs.

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METABOLIC FUNCTIONS OF THE ENDOCRINE SYSTEM

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This paper reviews some of the work published between September, 1941 and May, 1942. It has not been possible to consult the European journals, and only part of the North American ones have reached us.

The Pituitary

It is extremely difficult to establish the role of the anterior pituitary in different functions and the number of its hormones. In a strict sense all hormones have metabolic functions since they always produce changes in mass or chemical or energy modifications which can either be general to the whole organism or localized in a given organ. The hormones which have been isolated from the pituitary are generally not absolutely pure and may contain impurities which strengthen or weaken their action, or even produce opposite effects. The pituitary hormones act either directly or through other organs (such as the adrenal cortex, thyroid, and gonads). It is difficult to know the exact number of hormones secreted, since the process of extraction may divide some into several parts. It is not only of interest to know the action of the various substances isolated, but also which are really secreted and act as hormones; in order to establish this, it is always necessary to compare the results of pharmacological with those of physiological experiments and with the changes due to increased and decreased function. Moreover, administration of anterior pituitary may leave a permanent alteration after suspension of treatment.

The hormones of the pituitary have been the most studied during the time covered by this review. The greatest advance seems to have been made with prolactin. Li, Lyons & Evans (1) have described a product which they consider pure on the basis of the results of electrophoresis and solubility tests; it has a molecular weight of approximately 25,000 and contains 2.5 per cent tryptophane, 5.7 per cent tyrosine, 3.0 per cent cystine, 8.31 per cent arginine, and 1.79 per cent sulphur. White, Bonsnes & Long (2), starting with the hormone prepared by Lyons' method (3) after

a preliminary purification, were able to crystallize it using a mixture of pyridine and acetic acid or precipitating with acetone in an acid medium (pH 5.4 to 5.5). The product was found to be homogeneous by ultracentrifugation, electrophoresis, and solubility tests; it contained 5.5 per cent tyrosine, 1.3 per cent tryptophane, and 3.36 per cent cystine. It is sensitive to heat, pepsin, and trypsin; its activity is 30 to 35 I. U. per mg. Prolactin is sensitive to substances with sulphydryl groups. Fraenkel-Conrat, Simpson & Evans (4) found that cysteine in high concentration produced a true activation. In low concentrations it caused the hormone to change to a less soluble state; this when redissolved had the original activity. Thioglycollic acid inactivated at a lower concentration than did cysteine. Under these conditions Fraenkel-Conrat (5) has established that protein disulphide groups are reduced and, moreover, that reducing groups of an unknown nature appear.

The action of cysteine and other reducing substances on the gonadotrophin of mare's serum has been newly studied by Evans, Nelson & Cartland (6) who have found that cysteine and potassium cyanide produce inactivation; hydrogen sulphide produces only a partial effect in a solution of 40 per cent urea.

Gurin (7) has studied the carbohydrates present in the gonadotrophins and found that those in the anterior lobe of the pituitary contain mannose and hexosamine in equimolecular proportions, while those of human urine and mare's serum contain galactose and hexosamine in a molecular ratio of two to one.

The purification of the growth hormone has been studied by Abraham (8) in Evans' laboratory. He eliminates calcium with sulphur dioxide and has been able to establish that the protein fractions which are precipitated before the pH reaches 5.2 have very little activity; those precipitating at pH 5.7 to 6.2 have the greatest activity.

The discussion on the individuality of the oxytocic and vasoconstrictor principles of the posterior pituitary seems to incline towards Abel's opinion that, although with the methods employed substances with different activities can be separated, the original hormone is a single substance with several activities. By slightly acid extraction of dried posterior lobe of frozen gland and precipitation of the extract with salts, Van Dyke, Chow, Greep & Rothen (9) have obtained a protein which behaves as a homogeneous sub-

stance according to the results of electrophoresis, ultracentrifugation, and solubility tests. It has a molecular weight of 30,000, its isoelectric point is 4.8 to 4.9, and it contains 4.3 per cent cystine. It has multiple biological activities: it produces uterine contraction, it has depressor activity in fowls and pressor activity in cats and dogs, and it inhibits diuresis in rats. Reduction with thioglycolic acid almost abolishes its activity. The measurable activities (oxytocic action, inhibition of diuresis, and vasoconstrictor action) are present in the same proportion as in the standard posterior lobe powder of the American Pharmacopœia; one unit of these activities is approximately represented by 10μg. of protein nitrogen. On the other hand the melanophore-dilating action is much less. The authors suppose that the methods now employed to obtain the different principles separate the active parts of the original protein.

A new method for the separation of the oxytocic and vasoconstrictor activities based on their differential adsorption by zeolite has been described by Potts & Gallagher (10) who have obtained an amorphous powder with an oxytocic activity of 700 I. U. per mg. and less than 20 I. U. vasoconstrictor activity. It contains 18.3 per cent cystine, 14.2 per cent tyrosine, and less than 0.8 per cent arginine. The constrictor principle has an activity of 450 I. U. per mg. and less than 40 I. U. oxytocic activity. It contains 19 per cent cystine, 11.9 per cent tyrosine, and 12.3 per cent arginine, in contrast with the former.

The chemistry of the melanophore-dilating hormone has aroused new interest. Landgrebe & Waring (11) have described a method of preparation which gives a product almost entirely free of active principles of the anterior lobe, while Calloway, McCormack & Geiling (12) have proved that, as the alkalinity of the melanophore-dilator solution increases until pH 8 is reached, there is an increase in its potency, which they attribute to an effect on the molecule and not to the destruction of an inhibiting factor. By controlling the pH of the solutions Calloway, McCormack & Singh (13) have found that the hormone can be measured by its melanophore-dilating action in hypophysectomized frogs.

The anterior hypophysis of rats treated with 25 mg. of thyroid per 100 gm. body weight per day for 20 to 30 days acquires the power of depressing the thyroid, according to Reforzo-Membrives (134). Implantation in the guinea pig causes a decrease in weight

of the thyroid, flattening of the epithelium, and decrease in the basal metabolism of the animal. On the other hand, implantation of the anterior lobe of normal pituitary produces opposite results.

Role of the Liver and Pancreas in Carbohydrate Metabolism

Liver.—Foglia (14) has confirmed in the toad, *Bufo arenarum*, the indispensable role of the liver in the maintenance of a normal blood sugar level and in the production of hyperglycemia; this animal can survive total hepatectomy for six days. Total removal of the liver is rapidly followed by hypoglycemia; it also prevents hyperglycemia after pancreatectomy and after treatment with anterior hypophysis. The severity of pancreatic and hypophyseal diabetes is slightly reduced by removal of 55 per cent of the liver and considerably reduced when 75 per cent of the organ is extirpated.

Pancreas and insulin secretion.—Pancreatic endocrine function has been studied from several points of view: (*a*) effect of various hormones on the insulin content of the pancreas; (*b*) secretion of insulin in health and in diabetes; (*c*) the role of insulin.

Fraenkel-Conrat, Herring, Simpson & Evans (15) have confirmed that the implantation of pellets of α-estradiol dipropionate increases the content of insulin in the pancreas. They attribute this to an effect on the hypophysis because the implantation of the hypophysis of treated rats into another animal increased the insulin content of the pancreas, while the normal hypophysis did not. Moreover, estrogens did not increase the insulin content of the pancreas in hypophysectomized rats. Thyroxine (16) decreased the insulin content in the pancreas of hypophysectomized rats and increased it in normal animals. Hypophysectomy did not modify the insulin content of the pancreas in females and increased it in males up to the level found in females. Adrenalectomy also increased the insulin content. The purified lactogenic hormones of the hypophysis increased the insulin content; the growth hormone reduced it; the adrenotropic hormone sometimes increased it because of contamination with the lactogenic hormone; the pancreatotrophic action of the lactogenic hormone was seen in normal and hypophysectomized rats; the action of the growth hormone was not seen in hypophysectomized rats (17).

Insulin can improve both anatomically and functionally the

islets damaged by anterior hypophysis extract if the lesions have not advanced too far. An excess of insulin can produce anatomical and functional regression of the islets just as other hormones (such as those of the gonads and thyroid) affect the glands that secrete them. Mirsky, Nelson, Elgart & Grayman (18) have maintained dogs in permanent hypoglycemia by means of protamine-zinc-insulin after removal of 30 to 75 per cent of the pancreas; they found that twenty to forty weeks after beginning the treatment hyperglycemia and glycosuria developed and a permanent diabetes was established on discontinuing the insulin. No normal islets were found; only a few fibrosed ones were seen. They deduce that it would be dangerous to use protamine-zinc-insulin in the prophylaxis of human diabetes.

The regulation of insulin secretion has been reviewed by Houssay (19): blood sugar level regulates the secretion of insulin, and the secretion of insulin maintains a normal glycemia; the chemical factor is the principal and essential mechanism, the nervous (vagal) factor is secondary and dispensable; it only acts as an accessory mechanism increasing the speed of the adjustment.

In pancreatic diabetes there is a decrease in hepatic glycogen and a slow resynthesis of the muscular glycogen after exercise; the cardiac glycogen is increased; the glycogen in the leucocytes is also increased in pancreatic and hypophyseal diabetes (20). Evans (21) found that injection of glucose increased the glycogen in the normal heart; insulin did not increase it above the level reached with glucose alone; insulin, therefore, does not seem to be a necessary factor for the fixation of glycogen by the heart muscle.

Gellhorn, Feldman & Allen (22) have continued to study the insulin content of blood; they have found it present in the blood of normal dogs, but not after pancreatectomy except when insulin had been injected.

Anterior Hypophysis and Carbohydrate Metabolism

One of us (Houssay) has published three reviews on this subject: the history of the evolution of our knowledge of the role of the hypophysis in carbohydrate metabolism during the last twenty-five years (23); the history of hypophyseal diabetes (24); and the relations between the hypophysis and the pancreas (29).

Action of the hypophysis on the pancreas and on insulin secretion. —Houssay, Foglia & Smyth (25) have shown that only a small

amount of pancreatic tissue is necessary to maintain a normal blood sugar, but in an emergency the capacity to secrete insulin is always considerably diminished when the pancreas is reduced to one fifth of its normal weight; however it may be diminished or normal if half the pancreas remains. This diminished capacity was shown by (*a*) the slower return to a normal blood sugar level after injection of one gram of glucose per kilogram intravenously; (*b*) the smaller amount of hypophyseal extract needed to produce diabetes; (*c*) the effects of vascular grafting in diabetic animals. A normal pancreas connected by its blood vessels with a pancreatectomized dog reduces the blood sugar of the recipient to a normal level in three to five hours; a pancreas reduced to half its size does so within six hours only in eight out of thirteen cases; when reduced to one fifth it did not reduce the blood sugar to normal in nine out of ten cases. Methods (*b*) and (*c*) were more adequate to judge the emergency insulin secretion capacity than method (*a*).

Houssay, Foglia, Smyth, Rietti & Houssay (26) have confirmed and extended Houssay & Foglia's findings of 1936 that in hypophyseal diabetes there is a decrease or even suppression of insulin secretion. The pancreas of an animal treated with anterior hypophyseal extract for three or more days, when grafted, was less efficient in reducing the hyperglycemia of pancreatectomized dogs in fourteen out of seventeen experiments. Those animals that had a permanent hypophyseal diabetes showed severe damage in the islets and the insulin secretion was practically nil. In fourteen dogs resistant to the diabetogenic action of anterior pituitary extract, as shown by the occurrence of little or no change in blood sugar, the pancreatic secretion of insulin was normal in six cases, supernormal in three, and subnormal in five cases. In six resistant animals a high blood sugar appeared only on the day of the transplantation, but insulin secretion was normal in four, supernormal in one, and subnormal in one case. In these animals the beta cells were normal or hyperactive. With one day of hyperglycemia the capacity of the pancreas to secrete insulin was not altered. When hyperglycemia existed on the second to fifth day but fell later, the insulin secretion was normal in two, above normal in one, and less then normal in two. Histological examinations showed a restoration of the beta cells.

Anterior hypophyseal hyperglycemia is due at first to extrapancreatic factors which are the most important; they last only

during the injections of extracts. Pancreatic factors appear afterwards and are responsible for the permanent diabetes. Hypophyseal extract produces histological changes in many tissues and also damages the islets of Langerhans. The coexistent high blood sugar probably exhausts the beta cells and exaggerates their injury. In all cases there is a relation between the cytology of the beta cells and the insulin secreting capacity as shown by Porto (28).

In hypophysectomized dogs the pancreas is not diminished in weight; there is a marked increase in the islet mass with normal or hyperactive beta cells (28, 29). The capacity to secrete insulin is normal as judged by the effects on the blood sugar of pancreatectomized dogs when grafted (26, 29). These facts are not in agreement with the idea that there is a physiological pancreatotrophic activity of the anterior hypophysis.

Hypophyseal and Metahypophyseal Diabetes

The diabetogenic activity of the anterior hypophysis has been studied comparatively in different species; the test animals used are the dog with a pancreas reduced to four grams or the toad without pancreas and hypophysis (30). The human anterior hypophysis was found to be the most active and produced diabetes even in dogs with an intact pancreas. The other species came in the following order of decreasing activity: dog, toad, rat, guinea pig, fowl, ox, and, much less active, snakes and fishes. Corticosterone, desoxycorticosterone, prolactin, and the adrenotrophic hormone did not show a diabetogenic activity.

Hypophyseal diabetes should be differentiated from metahypophyseal diabetes. The first is observed during treatment with anterior hypophysis extract and is accompanied by hyperglycemia, glycosuria, ketonuria, hyperlipemia, fatty liver, and marked insulin resistance. It is at first an extrapancreatic diabetes to which later is added the insufficiency of the pancreas. Metahypophyseal diabetes follows discontinuation of treatment with anterior hypophyseal extract, and is really a pancreatic diabetes due to wide or total destruction of the islets. The extract produces a hypophyseal diabetes and progressively damages the islets; when the injections of the extract are discontinued, a metahypophyseal diabetes remains, so-called because it is has been produced by the effect of

the hypophyseal extract, but it is no longer a hypophyseal diabetes because the diabetogenic substances are no longer acting.

Lukens & Dohan (31) have studied in cats with a reduced pancreas the permanent diabetes produced by us in dogs in 1932. The glycosuria provoked by the anterior hypophysis extract is not seen when the animals are fasting or on a fat diet. Thyroxine and the thyrotrophic hormone do not produce glycosuria, nor does thyroidectomy modify pancreatic diabetes. The anterior hypophysis does not produce glycosuria in cats without pancreas and adrenals, but can produce a slight glycosuria in animals treated with adrenocortical extract. They deduce that the adrenals are necessary for the complete activity of the anterior lobe. Some of the animals survived six and nine months, and from the third month important lesions and atrophy of the islets could be seen; insulin treatment, and even phlorhizin treatment reestablished a normal blood sugar and improved the islets.

Ennor & Singer (32) confirmed in rabbits the findings of Dohan & Lukens in dogs (made in 1939) that treatment with diabetogenic anterior hypophysis extract provokes the appearance of antihormones which neutralize the diabetogenic effect. The Harrisons (33) observed in rats (as Biasotti did in dogs in 1934) a diabetic type of glucose tolerance curve twenty-four hours after injecting anterior hypophyseal extract, but as the treatment is continued the descent of the blood sugar is each time more rapid. The tendency to hypoglycemia during fasting in hypophyseal insufficiency has been confirmed in a human subject (34). In hypophysectomized rats neither thyroxine nor the thyrotrophic hormone prevents the rapid onset of hypoglycemia and depletion of glycogen stores which occur in fasting (35). These effects are much more pronounced if the hypophyseal stalk is amply resected; in this case adrenal atrophy and adiposity are also more marked (36).

Mechanism of the action of the hypophysis.—Russell (37) has observed that hypophysectomized, adrenalectomized, eviscerated rats need 25 to 30 mg. glucose per 100 gm. per hour while eviscerated controls only need 13.5 to 14 mg.; this increase in the need of glucose is not due to a larger storage of muscle glycogen or production of lactic acid but to a greater consumption of glucose by the tissues of hypophysectomized animals. Nephrectomy increases only slightly the requirement for glucose in the rat. Previous treatment with anterior hypophysis extract reduces the in-

crease in glucose consumption of the hypophysectomized eviscerated rat and reduces to two-thirds that of the eviscerated animals with hypophysis intact (38).

Foglia & Potick (39) injected 0.5 gm. glucose per kg. every half hour during five hours in dogs; the blood sugar levels rose higher in the hypophysectomized animals than in the controls (confirming Biasotti's findings in 1934). When the animals had been fed for several days a diet rich in carbohydrate the glycemic levels were lower than when the diet had been lacking in carbohydrates. This difference in behavior according to the diet was seen in normal and hypophysectomized dogs, but according to Himsworth, not in the hypophysectomized rabbit.

Samuels *et al.* (40, 41, 42) studied the influence of diet in hypophysectomized rats in which the weight was maintained by stomach tube feeding; animals on a carbohydrate diet had a basal metabolism 23 per cent lower and their tolerance to insulin was lower than that of controls fed the same diet. With a fat diet basal metabolism was 30 to 35 per cent lower and the insulin tolerance the same as in the controls. The hypophysectomized animals on a fat diet had less glycogen in the liver than those fed carbohydrate, but they lost it less rapidly on fasting. The hypophysectomized rats fed a fatty diet had less liver fat and more body fat than the controls.

Influence of the Hypophysis and Other Glands on Growth and Protein Metabolism

Griffiths & Young (43) have found that tail growth in the rat is proportional to the increase in body weight and, therefore, is a satisfactory method of measuring the activity of hypophyseal extracts that promote growth. This method can not be used in rats whose growth has been stopped by implantation of pellets of stilbestrol because the tail continues to grow out of proportion with the body weight, but in this case body weight increases proportionally to the dose.

Fraenkel-Conrat, Simpson & Evans (44) have studied purified preparations of the six hormones of the hypophysis in hypophysectomized rats; they found that only the thyrotrophic hormone increased the absolute and relative weight of the liver; the growth hormone only produced a slight increase in the absolute and a

decrease in the relative weight. Due to the slower growth the lesions caused by fluorine appear later in the teeth of hypophysectomized rats than in the controls (45).

Laqueur *et al.* (46) have seen that an active thyroid extract has a growth effect in hypophysectomized rats equal to that of the anterior lobe extract; thyroidectomized rats grew less with the anterior lobe extract than with the thyroid extract; simultaneous administration of both extracts produced growth in the thyroidectomized-hypophysectomized rats. Marx, Simpson & Evans (47) found that the purified growth hormone produced a greater effect when the purified thyrotrophic hormone was also given, but that the latter alone did not have a growth effect.

It is well-known that anterior hypophysis extracts can increase protein anabolism and diminish blood urea and non-protein nitrogen, but it is still discussed whether this is a direct action or one produced through the pancreas or the adrenals. Paschkis (48) has shown that antuitrin G decreases blood urea and usually blood sugar in normal rats, in adrenalectomized rats maintained with sodium chloride, and in partially pancreatectomized rats; therefore, he maintains that this activity of the anterior hypophysis extract is not mediated by the adrenals or the pancreas. This increase in protein anabolism was seen in normal and phlorhizinized rats, but not in rats intoxicated with monoiodoacetic acid (49). In the dog this extract also produces retention of sulphur.

Young (50, 51) has shown that the same extract can stimulate growth and nitrogen retention or have a diabetogenic action according to the age of the animal or the condition of its pancreas. The increase in weight seen in many diabetic patients and in cats and dogs treated with hypophyseal extract may be the result of an increased activity of the anterior hypophysis balanced by an increased activity of the pancreatic islets. In intense diabetes, growth ceases in spite of the treatment being continued; in a less intense diabetes the extract may increase nitrogen fixation and stimulate growth.

Cuthbertson, Webster & Young (52) have seen nitrogen retention produced in rats on a restricted mixed diet by crude extracts of fresh anterior lobes from oxen and sheep or of acetone desiccated ox pituitary tissue, while the extract of gland desiccated in alcohol is almost completely inactive. In all these experiments,

the period of increased nitrogen retention under the influence of pituitary extract was followed by a period of excessive nitrogen loss.

Cuthbertson, Shaw & Young (53) observed that daily administration of a crude alkaline extract of ox anterior pituitary lobe to rats suffering from a fracture of one femur prevents the loss of body weight and excessive excretion of nitrogen and of creatine which is the normal result of such an injury. The same authors (54) mention that this extract has no significant effect on the total period required to heal wounds caused by the removal of circular disks of skin from the upper part of the thigh of rats.

The decrease in serum albumin and increase in serum globulin seen in 1938 by Goldberg in the hypophysectomized dog has been confirmed in the hypophysectomized rat by Levin & Leathem (55); the authors accept as an explanation for the increase in serum globulin the insufficiency in thyroid function, since it is also observed after thyroidectomy and can be prevented by giving thyroxine to the hypophysectomized animals. The decrease in serum albumin is attributed to an adrenal insufficiency because it is prevented to a large extent by the administration of corticoadrenal extract or desoxycorticosterone.

The specific dynamic action is not modified by hypophysectomy in the dog (as was shown some time ago by Artundo, Mazzocco, Braier & Gaebler) but is decreased in the rat; Reiss (56) has shown that in this animal the dynamic action of albumin is less intense six days after hypophysectomy and has disappeared by the thirtieth day. Growth promoting extracts reestablish it, but the thyrotrophic, adrenotrophic, and gonadotrophic hormones were found to be inactive. The thyrotrophic hormone increased the oxygen consumption but did not modify the specific dynamic action.

Progesterone (5 mg. per day during nine weeks) given to infantile rats accelerated growth of the body and also of the thymus; it had no influence on the adrenal, hypophysis, and thyroid and decreased the weight of the ovaries (57). Emmens (58) has seen that hair growth in rats is not modified by castration, thyroxine, androgens, and pituitary extracts, but is decreased by thyroidectomy and the injection of estrogens. In females, but not in males, a saline extract of their own hypophyses increased hair growth.

The Adrenal Cortex

The influence of cortical hormones and other steroids on carbohydrate and protein metabolism.—Further confirmation has appeared of the fact that in the absence of the adrenals there is a decreased capacity to maintain a normal carbohydrate level and to produce carbohydrates from endogenous precursors. Evans (59) found that the adrenalectomized rat as compared with controls loses more carbohydrate in the early hours of fasting, stores less glycogen when intravenously injected with glucose or glucose and insulin, and has a normal intravenous glucose tolerance. Langley (60) confirms previous work of Evans showing that anoxia increases liver glycogen in the rat only if the adrenals are present. In the adrenalectomized rabbit treated with anterior hypophysis extract, fat but not glycogen is deposited in the liver as in normal animals (61). Anoxia produces hypoglycemia in the adrenalectomized dog, but not if the pancreas has been removed (62).

Selye & Dosne (63) have observed that trauma-producing surgical shock fails to cause the usual hyperglycemia in the absence of the adrenals. Active cortical preparations administered during traumatic shock cause a significant rise in the blood sugar concentration and at the same time increase shock resistance. Experiments on albino rats indicate that continued treatment with high doses of desoxycorticosterone acetate markedly inhibits but does not completely prevent the adrenal cortical response during the alarm reaction. As a result of this inhibition the animal pretreated with desoxycorticosterone acetate is less resistant to nonspecific noxious agents, since desoxycorticosterone acetate itself does not raise the resistance but apparently interferes with the production of those cortical hormones which increase general resistance. The desoxycorticosterone treatment interferes with the normal glycemic response to nonspecific agents, probably because it disturbs the production of cortical hormones active in carbohydrate metabolism (64).

Kendall (65) has reviewed the metabolic activity of the steroids with an oxygen atom in C_{11}. Functional disturbances in diseases of the adrenal glands have been reviewed by Kepler & Wilson (66). Cortical extracts and progesterone increase hepatic glycogen in young rabbits; estradiol, testosterone, and desoxycorticosterone have no such activity (61). Studies on rat liver slices indicate that

the rate of synthesis of carbohydrate from pyruvate and *d*-lactate is markedly increased by adrenal cortical hormone (67).

That the lowered capacity for work of the adrenalectomized rat can by compensated by glucose infusion had been shown by Ingle & Lukens (135). Ingle (68) has now studied the action of the new steroids. High doses of progesterone allow adrenalectomized rats to increase in weight and work more than the nontreated controls, but they remain below the normal standards. Immediately after operation 17(β)-hydroxy-progesterone, 6-hydroxy-progesterone, and Δ^4-17a-methyl-D-homoandrostene-17a-ol-3,17-dione had no activity.[1] Neither did 6-hydroxy-11-desoxycorticosterone diacetate enhance performance, but in large doses it increased weight; it is more active than progesterone but less than 11-desoxycorticosterone acetate.

The diabetogenic activity of the anterior hypophysis can be observed in adrenalectomized dogs and toads, but not as easily as in normal animals (23). This proves that the anterior hypophysis has a direct action on carbohydrate metabolism, not mediated by the adrenals.

Ingle (69) confirms that the administration of 17-hydroxy-11-dehydrocorticosterone to normal rats which were forcibly fed with a high carbohydrate diet caused hyperglycemia, glycosuria, an increased excretion of non-protein nitrogen, a marked loss in weight, and testis and thymus atrophy.

Not much new work has been done on the action of corticoadrenal hormones on protein metabolism. Evans (70) has observed in the adrenalectomized and nephrectomized rat a disappearance from the blood stream of *dl*-alanine given intravenously similar to that occurring in normal controls. Fraenkel-Conrat & Evans (71) have observed a striking increase in liver arginase under the influence of adrenocorticotrophic hypophyseal extracts and of adrenocortical extracts.

Role of the estrogens.—Confirming their own work and that of others, Janes & Nelson (72) have seen an increase in liver glycogen of rats treated with stilbestrol; the effect is more pronounced in males than in females and in the castrates than in normal controls; muscle glycogen is not modified. After twenty days' treatment the

[1] The reader is referred to Ruzicka & Meldahl [*Helv. Chem. Acta*, **23**, 364 (1940)] for clarification in respect to nomenclature.

blood sugar and urinary nitrogen increased, and there was indication that formation of carbohydrate from protein may be taking place. Lewis & McCullagh (73) have observed a decrease in glucose tolerance in rabbits and human subjects treated with methyl testosterone.

The administration of large doses of estrone, estriol, and diethylstilbestrol to four pituitary diabetic dogs and one depancreatized dog was found to exert no obvious antidiabetic action; in fact with estriol the effect was rather pro- than antidiabetic (74). The administration of stilbestrol to partially depancreatized nonglycosuric or mildly glycosuric rats on a constant medium carbohydrate diet produced a severe glycosuria and hyperglycemia in all cases (75). Similar results were obtained with dihydrostilbestrol, estradiol, and equilin. Massive doses of testosterone and methyltestosterone were weakly diabetogenic. The administration of stilbestrol to normal nonglycosuric rats on a constant high carbohydrate diet produced a mild temporary glycosuria in the majority of cases.

Epinephrine hyperglycemia.—The much discussed action of epinephrine on the rate of carbohydrate oxidation has been studied again by Conn, Conn & Johnston (76) in men by means of respiratory calorimetry. In normal fasting men a prolonged elevation of the blood sugar level, produced by epinephrine, is accompanied by a greater combustion of carbohydrate than is obtained at the usual fasting blood sugar level. Following the ingestion of a given amount of dextrose there occurs an increase in the rate of combustion of carbohydrate which is the same whether or not epinephrine is administered with the dextrose, despite the fact that the resulting blood sugar levels are widely different. With the same degree of hyperglycemia, approximately twice as much carbohydrate is oxidized when the hyperglycemia is produced by the ingestion of glucose as when it is produced by the administration of epinephrine. The level *per se* of the blood sugar does not control the rate of combustion of carbohydrate in normal men.

Influence of Thyroid Hormones on Carbohydrates and Protein Metabolism

In the thyroidectomized rat a moderate decrease in blood sugar and muscle glycogen has been observed; there was also a marked decrease in liver and heart glycogen (77). Injection of thyroxine for sixteen to twenty-two days produced liver damage with a de-

creased tolerance to galactose (78). Thyroxine and the thyrotrophic hormone do not produce glycosuria in cats with a reduced pancreas; thyroidectomy did not modify pancreatic diabetes significantly (31); thyroxine increased creatinine excretion in monkeys; testosterone suppressed this effect in both sexes (79).

Hormonal Influences on the Metabolism of Fat, Ketone Bodies, and Cholesterol

According to Heinbecker & White (80) obesity in dogs may be produced by partial destruction or retrograde degeneration of the paraventricular nuclei of the hypothalamus. Absence or decrease of the secretion of the neurohypophysis accentuates the deposition of fat accompanying the decrease in the cells of the caudal part of these nuclei.

Hyperlipemia and fatty infiltration of the liver have been seen in diabetic dogs fed fresh pancreas before discontinuing the insulin treatment, but not in those which had not received pancreas (81). Fatty infiltration of the liver can be prevented by giving 35 mg. choline per kg. or a fraction obtained by extraction of fresh pancreas. The choline content of this fraction is not sufficient to explain the lipotrophic effect. There is a thermolabile antifatty liver factor (82).

Soskin & Levine (83) have reviewed the problem of the formation of ketone bodies. They consider them as probably being formed in the liver as normal intermediate products of fatty acid catabolism. The liver is the principal if not the only source of ketones; they increase in the blood when there is excessive catabolism of fatty acids either through lack of carbohydrate or owing to faulty regulation of the production and consumption of carbohydrates. The effects of the anterior hypophysis, which increases ketonemia, and insulin, which diminishes it, are due to their action on the liver; neither of them produce these effects after removal of the liver.

Adrenal cortical extract, corticosterone, desoxy-1-corticosterone, and 11-dehydro-17-dehydroxycorticosterone (Kendall's compound E) do not stimulate ketosis in the fasting rat (84). Blood cholesterol is lowered in the rabbit by certain adrenal extracts (85). The thyrotrophic hormone diminishes blood cholesterol both in normal and thyroidectomized rabbits which have hypercholesterolemia (86).

Influence of Hormones on Biological Oxidations and Thermoregulation

Tyslowitz & Astwood (87) confirm that hypophysectomized rats do not maintain their metabolism at 0°C. The resistance to cold is increased by crude extracts of hypophysis and by corticotrophic hormone if the adrenals are intact, even if the thyroid has been removed. The anterior hypophysis, therefore, has a role in the protection against cold, mediated through the adrenal cortex.

The thyrotrophic hormone increases the oxygen consumption of thyroid tissue in Warburg's apparatus; thyroglobulin reduces it (88). Possibly thyroglobulin regulates thyroid function by a direct action. Oxygen consumption by the thyroid is increased by blood serum from acromegalic and myxedematous patients and depressed by serum from patients with Basedow's disease. The serum of some patients with Basedow's disease and dissociated ophthalmoplegia increased thyroid oxygen consumption.

The calorigenic action in rats of thyroglobulins containing from 0.046 to 0.38 per cent thyroxine is related to the thyroxine content and not to iodine content. The great variations in the thyroxine content and the constancy of other amino acids, especially of total phenolic acids, indicate that thyroglobulin is formed first and that further intramolecular transformation produces thyroxine (89).

De Meio (90) observed that epinephrine at 10^{-4} to 10^{-8} concentrations increased oxygen consumption by the retractor penis muscle in the Warburg apparatus. Concentrations between 10^{-9} and 10^{-11} produced a definite decrease in oxygen consumption; potassium cyanide inhibited this action of epinephrine by 70 to 100 per cent.

Influence of Hormones on Water and Salt Metabolism

Since the work of Ranson and his collaborators and of White & Heinbecker, the role of the diencephalic-hypophyseal mechanism that regulates the excretion of water by the kidney is being understood more and more clearly. Permanent or latent polyuria were not observed after separating the hypophysis from the hypothalamus in the cat or removing the hypophysis in the dog. Permanent polyuria was obtained immediately if on removing the hypophysis a drastic infringement on the hypothalamus was made; this would indicate the existence of an extrahypophyseal-anti-

diuretic elaborating mechanism in the vicinity of the anterior hypothalamus (91).

Macht & Bard (92) were able to maintain chronic decerebrate cats for 35 to 64 days; the sections were made at different levels between the caudal extremity of the diencephalon and the rostral limit of pons. In all cases a small portion of tissue remained consisting of hypophysis and hypothalamus, neurally isolated from the remaining brain stem, with its blood supply impaired as little as possible. This fragment could prevent polyuria in all cases where its blood supply was adequate.

The important role of the posterior hypophysis in maintaining blood pressure and capillary tone in batrachians, well-demonstrated by Orias in 1934, has not been the subject of further studies. Senderey (93) has seen a greater capillary permeability after total hypophysectomy but not after removal of the anterior lobe. This was shown in Laewen-Trendelenburg preparations which became more edematous and allowed a greater passage of water, salts, trypan blue, and congo red, but not of plasma proteins, into the tissues, than was seen in control preparations made with animals which had an intact hypophysis. Normal hypophysectomized animals are not edematous, but after destruction of the lymphatic hearts, there is a greater passage of fluid into the tissues than in the controls. This increased permeability can be corrected by previous treatment with posterior hypophysis, but not with anterior hypophysis. Schneider & Grant (94) have observed that hypophysectomy makes frogs more sensitive to water intoxication.

Polyuria produced by treatment with desoxycorticosterone and the antagonistic effect of posterior lobe extract have again been studied in the rat (95). Mulinos, Spingarn & Lojkin (96) have seen polyuria and polydipsia in five bitches which received 2 to 4 mg. daily of desoxycorticosterone during 12 to 180 days. At first water intake and weight increased, elimination of chlorides in urine decreased, the red cell volume and blood hemoglobin concentration diminished. After a short period of water retention, there was an increase in the volume and a decrease in the density of the urine. No weakening or toxic symptoms were observed. Oliguric action of pitressin was less than before treatment with desoxycorticosterone. They believe that in diabetes insipidus the corticoadrenal hormone is not neutralized by the secretion of the posterior hypophysis.

Hartman *et al.* (97) have announced the preparation of sodium-

retaining substances of the adrenal cortex that do not produce a refractory state. A method of standardization by comparing its action with that of desoxycorticosterone has been proposed (98).

For some time it has been known that in adrenal insufficiency the function of the kidney in the elimination of salts and water is faulty. Kottke, Code & Wood (99) have studied the urine dilution test; when adrenalectomized dogs were well sustained without cortical hormone on a high sodium-low potassium diet, as dilute or nearly as dilute a urine was obtained as from the intact animals. During hot weather they were unable to produce as concentrated urine as intact animals. The maximal chloride concentrating ability of the kidney was less than that of intact animals.

Wilson, Robinson, Power & Wilder (100) have studied a test for adrenal insufficiency in man. The administration of adrenal extract is discontinued and a diet containing 0.59 gm. sodium, 0.95 gm. chloride, and 4.1 gm. potassium per day is given; in addition 35 mg. of potassium per kg. are also given; on the following day 40 cc. of fluid per kg. and the same amount of potassium are given; on the third day 20 cc. water per kg. are given before 11 a.m. and the urine is collected from 8 to 12. Of the sixteen patients with Addison's disease studied, ten had symptoms of adrenal crisis, in five others there was a high concentration of sodium and chloride in urine, in one low blood sodium and chloride. In forty-four patients with normal adrenal function there was always a low sodium and chloride concentration in the urine.

Low potassium diets produce hypertrophy of the kidney, with dilatation, hypertrophy and hyperplasia of Henle's loop and the collecting tubules. Desoxycorticosterone produces this same hypertrophy in a less marked degree; it can be prevented by adding potassium chloride to the drinking water.

Progesterone and more markedly desoxycorticosterone reduce the creatinine and ascorbic acid clearance (by decreasing glomerular filtration) in rabbits (102).

Transfusion of blood serum to normal dogs or to adrenalectomized dogs treated with desoxycorticosterone is well tolerated; in nontreated adrenalectomized dogs it provokes circulatory collapse and edema, which disappear on injection of total adrenal extract. This is interpreted as due to an increase in capillary permeability which is corrected by the administration of adrenal hormones (103).

Parathyroids and Calcium Metabolism

The secretion of parathyroid hormone seems to be regulated by the calcium level, as hypocalcemia stimulates it. Hypocalcemia produced by injection of oxalate or transfusion of decalcified blood is followed by a rise in blood calcium in normal dogs, but not in thyroparathyroidectomized animals (104). This confirms Gerschman's (105) experiments with fluoride. However, the hypercalcemic curve after injection of calcium salts is similar in both normal and thyroparathyroidectomized dogs (106). A thyroparathyroid graft, made by connecting arteries and veins with the carotid and jugular of the recipient raises the blood calcium, lowers the blood phosphorus and suppresses the symptoms of tetany in thyroparathyroidectomized dogs; it does not alter blood calcium and phosphorus levels in normal animals (107). Patt *et al.* (104) perfused decalcified blood through the thyroids and parathyroids and subsequently injected it into normal dogs, which showed first an increase in blood calcium and then an increase in blood phosphorus. Normal blood perfused through the thyroids and parathyroids did not modify blood calcium and phosphorus in normal dogs.

It is still discussed whether the parathyroid extract influences calcium metabolism directly by stimulating osteoclasts or through its action on phosphorus. Neufeld & Collip (108) incline towards this latter view because parathyroid extract does not modify the blood calcium in nephrectomized animals nor in those with both ureters tied: in one cat blood calcium rose after cutting the ligatures on the ureter and reestablishing the flow of urine. Anuria produced by posterior pituitary extract does not produce hypercalcemia. Hypercalcemia can also be prevented by injecting acid sodium phosphate. These facts favor the hypothesis that parathyroid extract acts primarily on the excretion of phosphorus by the kidney, as is maintained by Albright (109), and not directly on the bone.

After injection of parathyroid extract and labeled phosphorus, there is an increase in phosphorus in the liver, kidney, and urine, but more labeled phosphorus is deposited in the controls than in treated animals (110). Williams & Watson (111) have seen that repeated injections of small doses of parathyroid extract temporarily increase phosphatase in the diaphysis but not in the epiphysis. Parathyroid extract increases blood calcium and phosphorus in hyperthyroid dogs as much as in normal dogs; thyroid

treatment does not increase urinary excretion of calcium in thyroparathyroidectomized animals as it does in normal ones (112).

Endocrine Glands and Iodine Metabolism

The effect of iodine feeding on thyroid activity has been studied by Ring (113). Iodine feeding decreases the metabolism of hyperthyroid subjects, but has little or no influence on the metabolism of normal men and animals. Nevertheless the treated rats survive only a few days when kept at 4°C., because they do not raise their heat production as do normal animals. Potassium iodide decreases metabolism only when there is loss of weight; when the iodine treatment is discontinued metabolism remains subnormal for three or more weeks.

The depressing action of iodine on the activity of the thyroid has once more been confirmed by Vanderlaan, Vanderlaan & Logan (114) by studies on the oxygen consumption *in vitro*. Guinea pigs treated with the thyrotrophic hormone show hypertrophy of the thyroid and an increase in the respiration of thyroid tissue; simultaneous treatment with iodine does not suppress the appearance of the hypertrophy but reduces the increase in oxygen consumption to a very small amount.

Several investigators have studied the role of the thyroid in iodine metabolism, including its fixation by the gland. Lein (115) describes an iodine tolerance test which consists in following the variations in blood iodine after giving a certain amount. In rabbits he gives 20 to 25 μg. per kg. and determines the iodine during three to four hours. After an initial rapid fall blood iodine remains stabilized for a relatively long time. When large quantities of iodine are injected in rabbits, the tolerance curve is not influenced by the hyperplasia produced by thyrotrophic hormone nor by removal of the thyroid; but when small quantities of iodine are given the basal level is reached earlier in animals with thyroid hyperplasia and later in thyroidectomized animals than in normal ones (116). The thyroid seems to have a limited capacity to fix a small amount of iodine; when large doses are given the amount fixed by the thyroid is not sufficient to have a visible influence on the tolerance curve.

Leblond & Süe (117) have also seen that small quantities of radioactive iodine are fixed by the thyroid; this gland fixes a greater proportion of iodine than other tissues, but it is neverthless

quickly saturated. They found 90 per cent of iodine in the form of iodide. The thyrotrophic hormone increases fixation of iodine and hypophysectomy reduces it; the hypophysis acts through its capacity of regulating the quantity and activity of thyroid tissue.

Perlman, Morton & Chaikoff (118) have continued their work on iodine metabolism using radioactive iodine; fixation and loss of iodine by the liver, muscle, and intestine, in the form of inorganic iodine, thyroxine, and diiodotyrosine follows the distribution of iodine in the plasma. The conversion of inorganic to organic iodine is quicker if thyrotrophic hormone is injected; thyroxine increases with time, not so diiodotyrosine. The thyroid of hypophysectomized rats fixes iodine much less rapidly; diiodotyrosine is formed and found in the thyroid and plasma, but its conversion into thyroxine is far below that of normal rats. In the plasma of normal rats 80 per cent of the radioactive iodine is found in thyroxine ninety-six hours after it was given, while in hypophysectomized rats only a small quantity was found in this state. It is concluded that the mechanism of diiodotyrosine formation is not damaged by hypophysectomy, but it cannot be synthesized into thyroxine (119). The differences of these authors' results with those of Leblond & Süe are explained by the fact that they did not use large quantities of iodine but only the small amounts necessary to act as indicators in the different fractions of iodine.

The thyroid can respond to iodine even when the hypophysis is missing; normal and thyroidectomized rats fed diets poor in iodine show an increase in thyroid weight, in height of the epithelium of the acini, and in vascularization of the thyroid (120).

Hertz & Roberts (121) have studied the action of the thyrotrophic hormone in rabbits given radioactive iodine. At first there is an increase in metabolism, relative weight of thyroid, and height of cells, followed by a decrease (except in weight), which is accelerated by the administration of iodine and which may reach subnormal values. The hormone at first stimulates thyroid growth and function; prolonged treatment and iodine administration provoke involution and functional depression of the gland.

Metabolic Action of Renin and Hypertension

In 1937 and 1938 Houssay, Fasciolo & Taquini (122) proved that the ischemic kidney of dogs with hypertension pours into the venous blood a vasoconstrictor and pressor substance. Braun-

Menendez & Fasciolo (123) confirmed these results and showed that acute ischemia of normal kidneys produces the same effect. Braun-Menéndez, Fasciolo, Leloir & Muñoz (124) have isolated from the venous blood of ischemic kidneys a substance, hypertensin, responsible for the pressor and vasoconstrictor activity. Later it was seen that this substance can be produced *in vitro* by the enzymatic action of renin on the blood globulins (hypertensinogen). Another enzyme found in blood and tissues, hypertensinase, destroys hypertensin (125). Ischemic kidneys secrete renin, not hypertensin; this last is formed in the blood by the action of renin on hypertensinogen (126). Hypertensinogen originates in the liver; it increases after nephrectomy and decreases after adrenalectomy.

Renin has been found in the renal venous blood (127, 128) and arterial blood (128) of dogs with hypertension due to renal ischemia. Experiments by Huidobro & Braun-Menéndez (129), Hamilton & Collins (130), and Sapirstein, Ogden & Southard (131) show that normal kidneys secrete renin when the arterial blood pressure falls. These results indicate a normal function of the kidney in blood pressure regulation through the secretion of renin.

Few experiments have as yet been made on the effects of renin and hypertensin on metabolism. Foglia & Moglia (132) have seen a rapid, marked, but transitory increase in blood potassium and a later and more prolonged rise in blood sugar in chloralosed dogs following intravenous injection of hypertensin. The hyperglycemia but not the rise in potassium was suppressed by adrenalectomy, removal of the liver, or injection of Fourneau 933.

Schaffer, Dill & Stander (133) also found an increase in blood sugar after renin injection; dalmatian hounds had an increased excretion of uric acid by 31 to 190 per cent and a rise in blood uric acid. When the injections were repeated during four days uric acid excretion increased 97 per cent over the initial amount.

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THE PHYSIOLOGY OF MAMMALIAN SEMEN

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In recent years, it has become increasingly apparent that male sterility is much more common than was once supposed. This realization has come as a result of the gradual establishment of more critical standards for the appraisal of human semen. Paralleling the interest in the more superficial aspects of the ejaculate (e.g., spermatozoa counts, motility, etc.), increasing emphasis is now being placed on the chemistry of the seminal fluid and the metabolic behavior of the spermatozoa. This review is concerned mainly with the latter topics since it is in these fields that the most recent advances have been made.

Cellular and Chemical Characteristics of Semen

While there is still much debate in regard to fertility standards for human semen, there is now available in the literature considerable data on semen quality in fertile and barren men. Meaker (1) has reviewed the problem of male infertility from a gynecologic viewpoint, particularly the question of the division of responsibility for sterile marriage between the male and female partners. More particularly, Hotchkiss *et al.* (2) have studied the semen specimens of two hundred fertile men and they consider that "an assay of fecundity includes a consideration of volume, grade of motility, number of spermatozoa, and percentage of abnormal forms." They found the average volume of ejaculate to be 3 cc. with a sperm count per cc. of 120.5 million. Twenty-five per cent of their cases had spermatozoa counts below 60,000,000 per cc. in spite of the evidence of Macomber & Saunders (3) who believe that pregnancy is not likely to occur with counts below that figure. Hotchkiss (4) could not find any definite seasonal variations in total spermatozoa counts but notes that in the month of April the semen specimens studied in his series of healthy men had the highest cell concentration. It is of interest to note that Erb *et al.*

(5) have recently reported that in bulls, the average concentration of spermatozoa and total sperm per ejaculate are at a maximum during April, May, and June.

In regard to conditions affecting the production of spermatozoa in the normal human, little is known. Wide variations are to be found in successive individual specimens (4) which cannot be attributed to illness or frequency of coitus. However, it has been found in the human (6) that elevation of body temperature, exposure of the scrotum to high environmental temperature, or both, results in a marked decrease in spermatozoa counts. This drop in count may not appear for eighteen days and the count may remain at a low level for approximately thirty-two days. These results are similar to those obtained by Moore (7) and others (8, 9) in laboratory animals.

The problem of differentiating between live and dead spermatozoa is usually decided by estimating the ratio of motile to nonmotile cells in any given specimen. This estimation is subject to considerable error but it has been estimated (6) that only about 60 per cent of the spermatozoa are motile in the average normal semen specimen. In a recent publication, Lasley *et al.* (10) describe a new staining technique for determining the number of live and dead spermatozoa in ram semen. They found that live (motile) spermatozoa were impermeable to certain stains whereas nonmotile cells stained readily. MacLeod (11) has applied this technique to human spermatozoa and found that the staining properties of the cells are reversible and cannot be correlated with the viability of the spermatozoa in so far as motility is used as a criterion of viability.

Chemistry of the mammalian sperm.—Until recently, studies on the chemistry of mammalian spermatozoa and seminal fluid (other than human) have been confined in the main to the metabolic exchanges between the cells and the fluid. The earlier studies have been reviewed in considerable detail elsewhere (12) and will not be considered at length here. More particularly, the chemistry of mammalian spermatozoa received little attention between the earliest studies by Miescher (13) in 1897 and the work of Zittle and his co-workers in 1941 (14). The latter took advantage of the sonic technique (15) to disintegrate bull spermatozoa (obtained from the epididymis) into their component parts (heads, mid-

pieces, and tails) and to analyze the various parts for certain chemical constituents. Large chemical differences were found in the various parts. The tails contained the greater part of the lipid (23 per cent) and the heads the largest amount of phosphorus (5 per cent), with all of the phosphorus accounted for as thymus-type nucleic acid. The sulfur content was also found to be high, ranging from 1.5 to 1.8 per cent in the various parts, most of it being accounted for as cystine and cysteine. The high cystine content in addition to the marked insolubility of the sperm in many reagents suggests the presence of a keratin-like protein, originally observed in fish sperm by Miescher (13). They found the sulfur and the phosphorus content of guinea pig and human sperm were approximately the same as in bull sperm. Green (16) observed that ram sperm contained a large amount of sulfur as cystine and his evidence also suggests the presence of a keratin-like protein. In a further analysis of the sulfur content of bull sperm, Zittle & O'Dell (17) determined the methionine content and found that the sulfur remaining after cystine was in the form of methionine. The mean value for the iron content is small (0.007 per cent), the mid-pieces and tails being considerably richer than the heads. The nonhemin iron was estimated to be 60 per cent of the total iron, (18) and since the cytochrome oxidase and cytochrome of bull sperm are concentrated largely in the tail (2), the suggestion is made that the mid-pieces and the tails are considerably richer in iron than the heads. By indirect methods, Zittle & Zitin (19) demonstrated the presence of the cytochrome system in bull sperm, the cytochrome oxidase content per mg. of tissue being about the same as that found in rat skeletal muscle. They also confirmed the presence of the succinic dehydrogenase. By far the greatest cytochrome oxidase activity was found in the mid-pieces and tails, the heads being metabolically inert in the respiratory sense. However, their statement that the sperm head "is probably a metabolically inert carrier of the chromosomes" should be taken with reserve since bull spermatozoa have a high aerobic and anaerobic glycolysis (admittedly a source of energy for motility independent of respiration) which possibly could derive from tissue devoid of hemin iron. In regard to other enzyme components, Lardy & Phillips (20) find the riboflavin content of bull sperm to be 30 μg. per gram of dry tissue.

Chemistry of Seminal Fluid

Perhaps the most thorough and comprehensive analyses of human seminal plasma have been made by Goldblatt (12) and by Huggins and his co-workers (21, 22, 23). These papers should be consulted for the earlier work on the subject but some of the more outstanding features of the chemistry are cited here. Normal human semen has a pH varying from 7.05 to 7.41. Goldblatt (12) gives the concentration of certain constituents of the seminal plasma as a ratio of their concentrations in the blood plasma as follows: chloride and cholesterol, 0.3 to 0.5; bicarbonate, 0.7 to 1.0; calcium, glucose, urea, 2.0 to 3.0; lactic acid, 5.0 to 6.0; acid soluble phosphorus (inorganic, 50 per cent; spermine, 30 per cent; undetermined, 20 per cent), 30. The proteins include: mucin (traces), nucleoprotein, albumin, globulin (traces), and proteoses. No peptone, protamines, histones, or proteolytic ferments are found but the presence of diastase and thrombokinase was demonstrated. The seminal plasma has a high buffering capacity principally due to the bicarbonate and to the enormous quantity of inorganic phosphate present (12, 24). Goldblatt's average value for the carbon dioxide content (equivalent to bicarbonate) is 22 m*M* per l. and this has been confirmed (23). It is well established that the glucose concentration of human semen is high, ranging up to 600 mg. per cent (12, 21, 25); it is of further interest that Goldblatt (12) finds the semen of diabetics to contain almost twice the amount of glucose found in normal semen. In regard to the contribution made by the various accessory glands to the semen, Huggins & Johnson (21) report that the secretion of the seminal vesicles contains approximately the same amounts of glucose, calcium, and acid-soluble phosphorus as semen and that spermatocele (epididymal) fluid and the prostatic secretion contain little or no glucose. Similarly, the seminal vesicle is the origin of most of the ascorbic acid (up to 3 mg. per cent) found in human semen (22) in which it is greatly increased compared to blood plasma. Seminal fluid contains lactic acid in large amounts (12, 21, 25). Its source and significance were in doubt until a recent study (25) on the fractionated human ejaculate made it plain that a much higher concentration of lactic acid is found in that part of the semen which, at the time of ejaculation, contains the greater mass of the spermatozoa. This analysis shows that, in the normal human

ejaculate, 75 per cent of the spermatozoa is contained in the first 40 per cent of the ejaculate. When the split ejaculate of azoospermic individuals (whose semen at no time contained spermatozoa) is similarly analyzed, the lactic acid concentration is found to be considerably lower than the normal and evenly distributed throughout the ejaculate. From this evidence, it is assumed that much of the semen lactic acid is derived from the *in vivo* metabolism of the spermatozoa, the remainder probably being contributed by the seminal vesicles.

In contrast to the distribution of lactate, the concentration of glucose in the second portion of the ejaculate is more than twice that found in the first (25) and this distribution is the same in the azoospermic ejaculate. In considering split ejaculates, it is of interest that Gutman & Gutman (26) found that human semen contains a very high concentration of "acid phosphatase." In analyzing the first, mid-, and last portions of the ejaculate, they found the first cubic centimeter to contain by far the highest concentration. It has been shown (21) that the prostatic secretion comprises the first part of the ejaculate proper and since the normal prostatic tissue is very rich in the "acid phosphatase" (27), Gutman's evidence would support the observation that the prostatic secretion precedes that of the seminal vesicles in the order of ejaculation. The physiological significance of the "phosphatase" is not known, the concentration of the substance in the semen of sterile males falling within normal limits. In regard to other chemical components, Schersten (28) reported a large citric acid content in human semen, most of which was contributed by the prostate and none from the seminal vesicle.

The proteins of human seminal plasma have recently undergone more intensive investigation. Huggins *et al.* (23) reported that the seminal proteins are largely proteoses (60 per cent). They found the average globulin content to be 1.2 gm. per cent, accounting for approximately 30 per cent of the total seminal proteins. Four protein fractions are observed electrophoretically in normal and pathological human semen (29). They have the same motility as albumin, alpha, beta, and gamma globulin found in blood serum and are presumed to be identical with these protein fractions. More complete electrophoretic and chemical data on the seminal proteins is given by Ross *et al.* (30). Their figure for albumin is

only about one-thirtieth of that given by Gray & Huggins and they have chemically separated a glycoprotein in electrophoretically homogeneous state which contains 11 per cent hexosamine and no uronic acid.

It is difficult at present to place emphasis on any particular aspect of the chemistry of seminal plasma. Enough evidence has been presented here to show that the seminal fluid has complexities beyond the only function yet known for it, namely, that of a simple carrier of the spermatozoa from the male to the female. If we assume that prior to ejaculation the spermatozoa have little or no motile activity and that motility is initiated by contact with the secretions of the accessory glands, then the high glucose content of the seminal vesicle secretion has an obvious function. This aspect of spermatozoa motility will be discussed later but it should be stated now that active motility can be maintained over long periods in a balanced salt solution, provided an adequate concentration of glucose is present (31). Whether the spermatozoa retain their fertilizing capacity under these experimental conditions is another matter and the question cannot easily be answered in the human. However, as will be shown later, spermatozoa of the bull and other animals retain their fertilizing capacity for extended periods when stored in various diluters.

Metabolism of Mammalian Sperm

That few studies on the metabolism of spermatozoa were made until recent years was undoubtedly due to the fact that sufficient uncontaminated experimental material was difficult to obtain. However, the widespread use of artificial insemination in farm animals required the development of techniques (e.g, the artificial vagina) for obtaining large amounts of uncontaminated semen and these, in turn, allowed reliable chemical analyses of the various semen constituents to be made. As a result, much data is now available on the metabolic behavior of the spermatozoa of most animals.

Metabolism of human spermatozoa.—One of the first investigations of the metabolism of human sperm was made by McCarthy *et al.* (32). They measured lactic acid production by incubating semen at 38°C. for periods ranging up to twenty-four hours. They analyzed the semen for glucose before and after incubation and

endeavored to correlate the sugar disappearance with the production of lactic acid. After twenty-four hours' incubation they found that the sugar concentration was only about 20 per cent of the original value, and though the lactic acid showed a marked increase it was far from enough to account for the sugar loss. Killian (33) performed similar experiments on human semen and showed that all the sugar lost during incubation could be accounted for in terms of lactic acid. On the other hand, Goldblatt (12) agreed with McCarthy *et al.* when he demonstrated that much more sugar was lost during incubation than could be found in the form of lactic acid. In all of these experiments, the authors did not allow for any complete oxidation of glucose which may have taken place. They also state that the bacterial growth in the incubated semen was negligible and, by implication, that bacteria contributed little of the metabolites under examination. It has since been shown (34) that, almost without exception, every specimen of human semen is contaminated with several types of bacteria and that even at room temperature, these bacteria grow rapidly. It should be noted, therefore, that it is hardly possible that twenty-four hour incubation experiments would not lead to serious errors due to bacterial growth. This is particularly true under aerobic conditions since certain aerobic bacteria may use lactate as substrate and this factor alone would account for the failure to find lactic acid in exact equivalence to glucose lost.

The first metabolic study of human spermatozoa using the Warburg manometric technique appeared in 1939 (35). The measurements were made in Ringer's solution and the metabolism was shown to be predominantly glycolytic, the aerobic glycolysis being only slightly below the anaerobic level. The respiration values were exceedingly small and it was shown that spontaneous loss of motility often occurred in the presence of oxygen. Shettles (36), however, reported that the respiration of human spermatozoa in seminal fluid was relatively high and varied inversely with the age of the specimen. He noted further that semen, devoid of spermatozoa, consumed no oxygen but it has since been shown (34, 37, 38) that most of the oxygen consumption of whole semen arises from the seminal fluid. More specifically, Zeller (38) states that most of the oxygen consumption of human semen can be attributed to the presence in the seminal fluid of a diaminoxidase

which uses spermine as one of its substrates. He finds none of the oxidase and virtually no oxygen consumption in the spermatozoa themselves.

Ross *et al.* (37), in experiments designed to determine if the metabolic characteristics of spermatozoa could be used as indicators of potential fertility, agree that glycolysis is the predominant feature of human spermatozoa metabolism and that prolonged exposure of the cells to oxygen depresses motility. They could not detect any quantitative differences in the glycolysis of spermatozoa from men of varying degrees of fertility, high and low figures being obtained from all groups. In a more detailed analysis of the enzyme systems concerned in the metabolism of human spermatozoa, MacLeod (34) could not find any spectroscopic evidence of the cytochrome complex, but he has since demonstrated (39) by other methods that the spermatozoa possess the complete cytochrome complex and also an active succinic dehydrogenase. If succinate be provided as substrate for the spermatozoa, a vigorous oxygen consumption takes place in spite of the fact that succinate alone will not support motility (39). Respiratory inhibitors such as cyanide, azide, and carbon monoxide do not depress motility (34) but the glycolysis inhibitors, monoiodoacetate and fluoride, inhibit glycolysis and destroy motility (31). Since motility is maintained for many hours under anaerobic conditions (34, 36), it seems clear that enough energy for motility is obtained from anaerobic mechanisms. The question of substrates utilizable for motility has also been investigated (31) and available evidence indicates that only certain carbohydrates (glucose, fructose, mannose, maltose, and glycogen) support motility. When motility is lost as a result of deprivation of substrate, it can be restored to a maximal level by addition of glucose, provided the cells have been left under anaerobic conditions throughout the course of the experiment (31). In regard to the toxic effect of oxygen upon motility, it is possible that, among other unique features of human sperm metabolism, these cells may produce enough hydrogen peroxide in the course of their metabolism to destroy motility. MacLeod (39) has shown that either hemoglobin or catalase will protect the spontaneous loss of motility which may occur in oxygen at 38°C. and that added hydrogen peroxide (1.5 to 3 parts in one million) will destroy motility rapidly. The suggestion is made that the peroxide is

formed through the autoxidation of a flavin-like enzyme and that the spermatozoa lack enough catalase to protect themselves against the peroxide.

Metabolism of other mammalian spermatozoa.—Much more data is available on the metabolism of the spermatozoa of lower mammals with most of the emphasis placed on the sources of energy for motility. Iwanow (40) measured the respiration of dog spermatozoa, and when he found that motility was maintained under anaerobic conditions and in the presence of respiration inhibitors, he concluded that the energy for motility did not arise from oxidative processes. Later (41) he showed that even when glycolysis was inhibited by monohaloacetic acid, motility persisted. He concluded, therefore, that neither respiration nor glycolysis furnished the energy for motility of dog spermatozoa. Lardy & Phillips (20), using similar techniques, come to the same conclusions for bull sperm though their evidence indicates that glycolytic and oxidative processes furnish energy for bull sperm motility under normal conditions. But when both of these mechanisms are blocked, motility persists for considerable periods of time. Using the Thunberg technique, they demonstrated the presence of an active succinic dehydrogenase in bull sperm but found that the methylene blue reduction time was greatly prolonged in the presence of glucose. The latter result is in direct contrast to the behavior of human sperm which reduce the dye very rapidly in the presence of glucose (39). Glucose inhibits the respiration of bull seminal spermatozoa but enhances the respiration of spermatozoa obtained from the epididymis (42). The latter point may be of considerable importance in that it indicates a definite change in the metabolic requirements of epididymal compared to seminal sperm, at least, in the bull. Phillips & Lardy (43), in experimenting with various diluters for prolonging the motility of bull sperm, found that an egg-yolk buffer pabulum would preserve maximal sperm motility *in vitro* for periods up to 150 hours if stored at 10°C. Sperm preserved in this fashion maintained their fertilizing capacity. The motility-maintaining substance or substances in the egg-yolk buffer have been shown to be phospholipids (44). Lardy & Phillips (44) measured the phospholipid content of bull sperm in the presence and absence of glucose and found that in the absence of the sugar the phospholipid content decreased about 40

per cent in ten hours at room temperature, whereas in the presence of glucose, no decrease was apparent. In summing up their results on the metabolism of bull sperm in relation to motility (44), they conclude that the energy for motility is obtained from the oxidation of intracellular substances (phospholipids) or from glycolysis. When sugar is available, the anaerobic mechanism is sufficient and lessens the demand on the oxidative mechanism. It is on this basis that they explain why the "endogenous" respiration is inhibited in the presence of glucose.

In regard to the glycolysis of seminal bull sperm, the figures given by Lardy & Phillips (20) for aerobic and anaerobic glycolysis are virtually identical and quantitatively similar to the figures for human cells. The results of Henle & Zittle (42) for the same cells, however, are very much higher and comparable to those given by Redenz (45). Furthermore, both of the latter authors find the aerobic glycolysis to be only about 55 per cent of the anaerobic.

Comstock (46) in a study of variations in the glycolytic power of ram sperm found a strong correlation between glycolysis and motility but Chang & Walton (47) in the sperm of the same species demonstrate a better correlation between respiratory activity and motility. On the other hand, Moore & Mayer (48) in a chemical analysis of ram semen found that good motility would continue for several hours after all the semen sugar had disappeared and their evidence indicates that a mechanism other than carbohydrate breakdown provides the energy for motility in ram spermatozoa.

In a series of papers on the effects of pH, cell concentration, etc., on the respiration of ram and boar sperm, Winchester & McKenzie (49, 50, 51) point out that at sperm concentrations from 1 to 6 billion cells per cc. an increase in concentration was accompanied by a decrease in respiration rate. They found the optimum pH for respiration of boar semen is 7.2 to 7.3 and for ram semen, 7.0 to 7.2. These papers are of further interest in that they show that sperm-free semen has an appreciable oxygen consumption which is not due to the presence of bacteria.

In regard to the effect of certain toxic substances on spermatozoa, Henle & Zittle (52) note that gramicidin (5 to 10 mg. per cent) first stimulated and then inhibited motility of bull sperm in Ringer-phosphate solution at pH 5.5 to 6.6. At pH 7.9 only stimulation occurred. In Ringer-bicarbonate at pH 7.4, gramicidin

decreased oxygen uptake 70 to 80 per cent and in all cases, glycolysis was partially inhibited. Shettles (53) has determined the resistance of human spermatozoa to concentrations of sulfanilamide and sulfapyridine *in vitro* well above the tissue concentration achieved by therapeutic doses and found that they do not affect the survival or activity.

Problems Relating to Artificial Insemination in Animals

The practice of artificial insemination is now widespread in the field of animal husbandry and new methods for prolonging the fertile life of semen are constantly being devised. The Russian school was responsible for most of the early developments in this field, particularly in devising various dilutors. Glucose-phosphate and glucose-phosphate-lecithin solutions (54, 55) were found to give good results in artificial insemination if the semen diluted in this fashion was not stored for any length of time (56). In 1940, Phillips & Lardy (43) developed a diluter which had as its base fresh egg-yolk dissolved in phosphate buffer at a pH of 6.75. When fresh bull semen was combined with the yolk buffer in the proportion of 1:3 or 1:4 and stored at 10°C., the fertilizing capacity of the spermatozoa was regularly maintained for periods beyond 100 hours. They bred cows successfully with yolk-buffered semen stored for 150 to 180 hours. Comparable results have been reported (57) with the use of yolk-citrate diluter. On the other hand, it was found by Underbjerg *et al.* (58) that various types of diluters, including egg-yolk buffer, had little beneficial effect in preserving the fertilizing capacity of bovine spermatozoa. It seems generally agreed that semen should be stored at temperatures lower than that of the body but temperature shock as a result of rapid cooling should be avoided (10, 47, 59). This subject has been analyzed in considerable detail by Easley, Mayer & Bogart (60) who agree that the yolk-phosphate diluter is superior to all others, particularly for the longer storage times. They found that the greatest sperm survival was attained when the storage temperature was lowered to 5°C. in successive steps, ninety minutes apart, of 5°C. Gunsalus, Salisbury & Willett (61) agree that the storage temperature should be 5°C. or lower in order to limit the tremendous bacterial growth which takes place at room temperature, and Easley

et al. (60) believe that the slower growth of bacteria at low temperatures may be the factor favorable in storage of undiluted semen at 5°C. They report further, that semen diluted with yolk phosphate is not influenced to the same degree as undiluted semen by the temperature at which it is stored. This paper should be consulted for data on the osmotic pressure, pH, and buffering capacities of the various diluters, and for a comprehensive bibliography of the entire field.

For a critical review of artificial insemination in livestock breeding, the comprehensive monograph by Lambert & McKenzie (62) is invaluable.

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ENDOCRINOLOGY OF REPRODUCTION

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The fundamental concepts concerning endocrine control of reproduction have not changed appreciably since the recent reviews (1, 2), but experimental data presented within the last year has clarified some of our ideas. Although the international situation has markedly reduced the number and availability of foreign publications, until now there has been no notable decrease in the number of publications in American journals so that the material to be reviewed is still very extensive. Since space is limited it will be necessary to omit many publications which do not fit well into the scheme of the review and merely to mention others which merit greater consideration. It is hoped that many of these will be reviewed in related chapters.

Gonad-Hypophyseal Relationships

Hormonal control of the gametes has been demonstrated as far back in the phylogenetic series as some of the more primitive insects (Orthoptera) where development of the ova and oviducts, but not of the spermatozoa, has been shown to be under the control of the corpora allata (3), which in certain respects resemble the hypophysis of the vertebrates. This control may, however, be through a metabolic hormone. Absence of the ovary is followed by hypertrophy of the corpora allata indicating an interaction of these organs. An analysis of this mechanism is awaited with interest.

Our analysis of the endocrine control of reproduction in the vertebrates has been based mainly on the common laboratory mammals, which often made us fail to appreciate some of the complexities of the endocrine system since these animals respond so readily to almost any endocrine manipulation. The establishment of gonad-hypophyseal interrelationships is essentially a maturity phenomenon. They can be permanently modified during the first ten days of postnatal development in the rat by testicular grafts or by the injection of either estrogens or androgens (4), but such

treatment applied after the fifteenth day yields only temporary effects. A certain degree of maturity is also required for the gonads to become responsive to gonadotropins (5, 6, 7), and the sensitivity of the accessory organs of reproduction is conditioned, at least in part, by the age of the tissue, the greatest increase in sensitivity occurring at puberty (8, 9, 10). The latter finding suggests that many of the changes at puberty are due to increased sensitivity to amounts of gonad hormone already present rather than to an increased secretion of hormone, as previously thought. The effects of sex hormones on the adrenals and the hypophysis also vary with age (11).

Hypophyseal gonadotropins.—One of the more significant changes in concept in recent years has been the realization that interstitial cell-stimulating hormone (ICSH) and luteinizing hormone (LH) are identical (12, 13, 14) and that they represent a single definite chemical entity (15, 16). That the protein which was isolated is the hormone was demonstrated by its biological activity remaining unchanged throughout purification by solubility, electrophoresis, and ultracentrifugation (15). However, ICSH obtained from swine appears to be a different protein from that separated from sheep hypophyses since it has a different isoelectric point, as shown by electrophoretic analysis (15). ICSH stimulates the interstitial cells of both the testis and the ovary and induces the production of male hormone in the male (17, 18, 19). The function of the follicle-stimulating hormone (FSH), which had until recently been better understood than ICSH (or LH), now seems to be relatively obscure since some of the effects attributed to it are now known to be due to its contamination with ICSH. FSH causes growth of follicles in the ovary and development of the seminiferous tubules in the testis without production of sex hormones (20). It is only when FSH is combined with a small amount of ICSH that estrogen is produced by growing follicles. Preovulatory swelling, ovulation, and corpus luteum formation require a level of ICSH much higher than that required for maintaining the interstitial cells in the ovary or testis (21).

The identity of ICSH and LH (12, 13, 14) requires the formulation of a new working hypothesis to cover the maintenance of interstitial cells under conditions which will not induce luteinization. Since a mixture of purified FSH and ICSH has been shown in hypophysectomized rats to produce predominantly follicle-

stimulating effects in females and interstitial cell-stimulating effects in males (22), it is evident that there is a difference in responsiveness of the two tissues to the same level of gonadotropic hormones. Thus, it appears that the hypophysis of the male differs from that of the female in secreting ICSH at a level which is possibly constant, but is lower than that required for luteinization of the follicles and cannot be raised sufficiently, even in the presence of estrogen, to be effective in this respect (21). Injected androgens inhibit the release of ICSH but increase the lactogenic content of the pituitary of the rat (23).

Considerable information concerning gonad-hypophyseal interrelationships has been obtained by study of the pituitary (24, 25, 26), especially with reference to colchicine mitoses following induction of various hormonal and environmental states in rats (27, 28, 29), monkeys (30), cats (31), and necturus (32). The prevention of castration changes seems to be an action common to all hormonally active steroids (33).

Estrous cycle.—During growth of the follicles both FSH and ICSH must be present (20) but there must be a sudden increase in ICSH at the time of preovulatory swelling, and the level of ICSH must remain high during ovulation and corpus luteum formation. The estrogen produced by the growing follicles is thought to be responsible for suppression of FSH and increase in ICSH. However, Heller *et al.* (34) have doubted that estrogen, even in amounts beyond the physiological range, decreases the potency of the rat hypophysis, but suppression of the release of gonadotropic hormones of the hypophysis by very small doses of estrogen has been demonstrated in parabiotic rats (35, 36). One to 4 μg. of estradiol will suppress the hypophysis of the castrate member sufficiently to allow the intact female parabiont to again run normal cycles without interference by the hypophysis of the castrate. This is not due to the transfer of estrogen inasmuch as forty times the minimal dose of estradiol must be injected for detectable amounts to cross over into the other member of the pair (36). Estrogen does not seem to increase the release of ICSH in male rats (21), but at least an effective level for luteinization is reached in the female. Once the corpus luteum is formed the ICSH has no further effect, either in activating it or in causing its involution (20, 37); the secretory function of the corpus luteum depends on another hormone which may (38, 39) or may not (40) be lactogenic hormone.

Follicular development in the immature rat is reported to be unaffected by injections of lactogenic hormone (41).

Because of the readiness with which ovulation can be induced in the common laboratory rodents, the findings on these animals have not contributed materially to the analysis of the intricate hormonal balance which exists in such groups as the birds and primates. In the fowl, although laying does not continue (42), the follicles can be induced to grow by equine gonadotropin (PMS), and ovulation can be produced 6.8 hrs. after intravenous injection of 4 r.u. of LH (43, 44). In laying hens follicles can be caused to ovulate as much as twenty-seven hours prematurely by administration of LH (45). A few instances of induced ovulation have been reported in the monkey (46, 47) but conclusive proof of this in the human is lacking, even though there have been sporadic reports of successful ovulation and successful responses in menometrorrhagia and ovarian sterility following administration of gonadotropins (48, 49). The results obtained from the use of gonadotropins in gynecologic disorders are at best difficult to evaluate (50 to 60) since it is not easy to obtain controlled experimental conditions. Follicles with multiple ova have been reported to be produced by PMS in the monkey (61). A combination treatment with FSH and LH was found to invariably produce ovulation in the anestrous opossum (62), and PMS has been reported to have been used successfully to evoke ovulation in horses but was not so effective in cattle (63).

Pregnancy.—Immediately following mating, FSH, ICSH, thyrotropic hormone, and perhaps adrenal-stimulating hormones, are released by the hypophysis of the female rabbit (64). These are presumably augmented by lactogenic hormone if the latter is responsible for activiating corpora lutea. Progesterone produced by the corpora lutea is essential to the formation of the maternal placenta (65) but will not maintain it indefinitely (66). Pregnancy has been maintained in rats hypophysectomized one to nine days after mating by injected lactogenic hormone or hypophyseal-gonadotropic synergist (FSH plus a small amount of LH) free of all but traces of lactogenic hormone (40). Under the latter condition new corpora lutea form and may possibly produce enough progesterone to carry over until the placenta is sufficiently developed to take over the maintenance of the corpora lutea, which in the rat (67) and mouse (68) may occur as early as the eleventh

day. No ICSH appears to be produced in the hypophysectomized pregnant mouse (68).

During ovulation the area of germinal epithelium bathed by the liquor folliculi undergoes mitotic activity (69) which increases the number of primary ova formed (70). This effect is presumably due to the estrogen from the liquor folliculi (71). In the opossum numerous ova are formed from the germinal epithelium during the anestrum or proestrum, but only occasionally does this occur as a result of cell divisions at right angles to the epithelium (72), as described in mice but found not to occur in guinea pigs (73). The passage of the ovum through the tube and into the uterus and the development of the embryo up to the time of implantation can occur in the absence of ovarian hormones (74, 75). In the weasel and martin the blastocysts from matings in late July and August remain dormant in the uterus until the following spring when they implant. It is not known whether there is any hormonal control of the blastocysts during the dormant period (76). There is a characteristic antimesometrial hyperemia during estrus in the guinea pig (77) which may have some relation to deciduoma formation. Deciduomata seem to develop best under an optimum balance of estrogen and progesterone, but the actual amount of hormone present is also very important (78). Deciduomata can be elicited by mechanical or electrical stimuli under the influence of progesterone alone, or in combination with estrogen, but they cannot be maintained indefinitely in either castrated or castrated and hypophysectomized mice (79) or castrated rats (66). Implantation may be a function of the uterine mucosa as well as of the blastocysts (80). The site of attachment seems to be definitely regulated, but the factors involved are unknown (81). The virgin uterus, when properly sensitized to produce pseudopregnancy, will support the embryo during the early critical stages, lasting up to twelve days in some cases (82). Endometritis prevents intrauterine but not ectopic implantation (83), and a full term abdominal pregnancy with survival of mother and child has recently been reported (84).

The placenta can effectively take over functions of the hypophysis, but the ovary is essential practically throughout pregnancy in the rat and mouse whose placentae apparently do not assume the functions of the corpora lutea (85). However, in primates the function of the ovary is fully taken over by the placenta. This occurs in the monkey by the time invasion by the trophoblast

is complete (86). Castration of the monkey on the twenty-fifth day of pregnancy does not interfere with pregnancy, parturition, postpartum involution of the uterus, or lactation. Daily injections of 1.0 mg. of progesterone beginning on the day of mating prevent implantation in mice (87). If begun on the second day, implantation occurs and the embryos are maintained as long as progesterone is supplied. Implantation is delayed in lactating rats. This effect is attributed by Weichert (88, 89) to a deficiency of estrogen resulting from its removal in the milk. Krehbiel (90), however, has shown that the implantation sites formed when estrogen is injected into lactating rats are not maintained in the post-implantation period even when large amounts of estrogen are injected. Prolonged gestation of lactating rats is apparently the result of delayed implantations, shown by Krehbiel (91) to occur at regular intervals separated by periods of approximately the length of the estrous cycle. The post-implantation period is always 16 (± 1) days in the rat regardless of the length of pregnancy. It has been reported that the deciduomal reaction requires a sensitization of the endometrium different from that required for induction of the decidual reaction (92).

Little is known of the possible hormonal control of parturition although histologic changes in the endometrium throughout pregnancy have been described for the rabbit (93). Large amounts of progesterone are essential during the post-implantation period in the rat, and estrogen is also present during this period, but the level is not believed to be an important factor in parturition (94). In rats hypophysectomized before the placenta can take over, pregnancy may be maintained by lactogenic hormone and may even be carried beyond term, a fact suggesting that cessation of production by the placenta of the substance which maintains the corpora lutea may be a factor in the control of parturition. Any part estrogen or progesterone may play is not reflected by changes in the excretion of estrogen and pregnandiol (95). Instances of probable superfetation occasionally occur in rodents (96) and may possibly be explained on the basis that occasionally some blastocysts remain unimplanted at the normal implantation period and become implanted at a later time (97). At parturition there is a discontinuation of suppression of the pituitary gland since ovulation in the rat occurs on the average of 28.5 hours after parturition,

but depends somewhat on the time of day at which the young are born (98).

Injection of gonadotropic substances.—The gonadotropic substances are classified as pituitary gonadotropins (of hypophyseal origin), chorionic gonadotropins (from the placenta), and equine gonadotropin (PMS). That these substances are different in action (99 to 102) as well as different proteins is well established. A start on an analysis of the structure of the chorionic and equine gonadotropins (103, 104, 105) has been made, but further study will be necessary before the physical and chemical properties of these substances are well known. Preparations of castrate urine are primarily follicle-stimulating in action. Pregnancy urine preparations (PU) are primarily luteinizing or interstitial cell-stimulating in action in the common laboratory animals (106) but depress the ovaries of the primates (60). There seems to be some question as to the ratio of FSH to ICSH in the normal male urine (107, 108, 109), as well as in the hypophysis (110), but there is no increase in the output of gonadotropins in old age, a fact indicating that the testes of old men still maintain the interrelationship between the hypophysis and gonads (111).

In any analysis of gonadotropic potencies it is important to bear in mind the differences in the responses of various strains of rats (112) and mice (113). The procedure employed in preparing the material to be tested may also affect the assay values. Air drying of pituitary glands causes the least loss of activity (114), and serum of pregnant women may be reduced to a powder without loss of activity (115). Minced suspensions and divided doses make it possible to get a positive gonad response with a single male rat hypophysis (116). PMS is augmented by FSH but not by chorionic gonadotropin (117), and synergism may not depend upon any specific gonadotropic action (118). The testes of hypophysectomized animals may be maintained or restimulated by PMS in rodents, including the guinea pig (119), but only for a short time in monkeys, presumably because of the formation in the latter of antisera (120). The potency of the anterior hypophysis varies with the age and sexual condition of the animal, as illustrated in rabbits (121) and fowl (122, 123). The hypophysis of the cock is seven times as potent as that of the nonlaying hen (122) and has a relatively high LH content (123) although the function

of LH is uncertain, at least in the dove (124). Insults to the female hypophysis, such as that resulting from intravenous injection of near lethal doses of copper salts, not only cause ovulation but induce pseudopregnancy in the adult estrous rabbit (125). This action may be inhibited by progesterone or pituitary stalk section (126). The gonadotropic activity of plant juices is probably also due to insult to the pituitary gland inasmuch as these substances are only effective in causing rabbits to ovulate during the season of greatest responsiveness (127). The sex maturity factor of orally applied plant juices is not so readily explained (128).

Thyroidectomy decreases the gonadotropic potency of the hypophysis of the male mouse, presumably by suppressing FSH (129). In rats gonadotropic hormones are much more effective following thyroidectomy (130). There also appears to be a close relationship between gonadotropic hormones and ascorbic acid, gonadotropic hormones giving greater stimulation when combined with ascorbic acid than when alone (131); and large doses of PMS cause a marked decrease in the ascorbic acid content of the blood plasma of male and female cattle (132). It is also decreased in castrated cattle, in which, interestingly, recovery is much more rapid (133). Injections of epinephrine are reported to suppress the function of the testes of rats (134), while acetylcholine and eserine are said to enhance the response of rabbit ovaries to chorionic gonadotropin (135). An acetylcholine-prostigmine mixture applied directly to the pituitary gland of the rat results in pseudopregnancy, while atropinizing the exposed hypophysis prevents the pseudopregnancy which follows electrical stimulation of the uterine cervix; these findings indicate an acetylcholine-like humoral transmission between the hypothalamic region and the anterior hypophysis (136), which would explain the nonessential nature of the nervous connections in the pituitary stalk (136, 137) or greater superficial petrosal nerve (138).

The exteroceptive stimuli which alter or regulate the gonadotropic activities of the anterior pituitary (139) and the factors controlling mating behavior in females (140) have recently been reviewed and therefore will not be discussed here. However, more recently reported observations on the effects of light in stimulating the testes of the starling should be mentioned. It has been shown that one second of light is required for illumination to affect the activity center, but this stimulus must be repeated every fifteen

seconds to provoke the release of gonadotropic hormones from the hypophysis since the stimulus apparently fades out after fifteen seconds of darkness (141). Evidence is still being accumulated against the idea of zoological specificity of the pituitary hormones (142).

Antihormones

Antihormones have recently been reviewed (143, 144), but additional work having a direct bearing on the endocrine control of reproduction has been published since these reviews appeared. Injections of antigonadotropic hormones inactivate the gonadotropic hormones released by the hypophysis, causing the gonads to regress with the result that there are no sex hormones to act back on the hypophysis, and typical castration changes occur in the hypophysis (145). For this reason precocious sexual development may occur in rats subsequent to a short period of treatment with antigonadotropic hormone (146, 147), and corpora lutea may be caused to form in the ovaries of persistently estrous rats by the same treatment (148). This relationship may also explain the increased response of the prostate after cessation of gonadotropic hormone treatment (149). By subjecting a relatively pure fraction of FSH to tryptic digestion and injecting the more purified hormones into rabbits, a pure anti-FSH hormone can be obtained, by use of which it is possible to demonstrate that equine gonadotropin (PMS) contains two hormones (150). It seems unlikely, however, that the antisera actually destroy the gonadotropin since prolan can be freed from a mixture of prolan and antiprolan by destruction of the antiprolan with 0.1 N NaOH (151).

With the relatively crude gonadotropic hormone preparations, at least, it appears that the specificity of the antihormone depends on the protein source of the gonadotropin; antiprolan is only slightly effective against gonadotropins other than chorionic (152), and sheep antigonadotropic hormone is not effective against gonadotropins of the chicken and vice versa (153). The same antigens are observed in ox pituitary preparations as in analogous fractions of ox kidney and are probably protein in nature (154). However, interstitial cell-stimulating hormone isolated in a pure state from hog hypophyses is antigenic, but its antiserum not only is species-specific but does not react with other hormones from the same lobe of the hypophysis (155). Evidence for the existence

of two antibodies for crystalline insulin has been presented (156).

It may be mentioned that estrogens are reported to evoke an increase in nonspecific antibodies (157), and when administered with pneumococcal vaccine cause a remarkable increase in protective antibodies (158). Estrogens also increase the bactericidal properties of blood serum of rabbits against *E. typhi* (159). Both pregnancy and sex hormones affect resistance to infection (160).

Menstruation

Menstruation remains poorly understood in spite of a vast amount of study. The spiral arteries are about the only anatomical feature which were still thought to be responsible for menstruation (161, 162), but even they are now found to be unnecessary (163). The prerequisites for menstrual bleeding may be entirely physiological and not dependent upon any anatomical peculiarity of the primate uterus (163). Menstruation will follow the discontinuation of administration of either estrogen or progesterone. After the uterus has been sensitized by estrogen, menstrual bleeding in monkeys will follow the cessation of administration of doses of progesterone as small as 1.0 mg. daily for one to five days even though estrogen administration is continued. Omitting estrogen treatment for two days after twenty days of treatment does not precipitate menstruation. On the other hand, when estrogen is administered for a period of several weeks, bleeding will occur two or three days after discontinuation of treatment. This fact indicates the endometrium has now become as sensitive to estrogen deprivation as to deprivation of progesterone although the development of sensitivity to deprivation of the former requires much more time (164). Although estrogen augments the action of progesterone, the resultant progestational changes in the uterus are entirely different from the normal response to estrogen. For this reason the progestational changes cannot be maintained by estrogen alone (164).

The elephant shrew has been reported to have a menstrual process similar to that of the primates, with the exception that while the entire endometrium exhibits the estrogen phase, only the mesometrial side shows a luteal phase and bleeds (165). It has been shown in intact baboons that it is possible by means of estrogen and progesterone injections to alter profoundly the ovarian cycle without changing the menstrual cycle, this fact indicating

that a normal interval between menstrual bleedings is not necessarily a sign of a physiologically normal cycle (166). Placentation in the Jamaican bat (167) and the antelope (*Rhydchotragus kirkii nykae* Heller) (168) and the natural circulation in the thirteen day human (Torpin) embryo (169) have recently been described.

Physiology of Sex Hormones

Mode of administration.—Physiologically active sex hormone compounds are too numerous to be discussed completely in this brief review. It is sufficient to say that the chemist is so far ahead of the biologist and clinician that almost any possible preparation is available in synthetic form, and much attention is being given to the best methods of administration. Pellets give the most uniform absorption and involve the least amount of work. If a hormone is absorbed too rapidly, it may be mixed with cholestrol in various proportions (170) or injected in paraffin (171), the only disadvantage being that the amount of hormone absorbed after any given period of time cannot be determined. The rate of absorption and some of the influencing factors have been ascertained for uniform pellets of pure desoxycorticosterone acetate, estriol, androsterone, α-estradiol, α-estradiol dipropionate, and α-estradiol benzoate, here arranged in the order of most rapid absorption in the rat (172, 173) and presumably in the human (174). In the guinea pig the same number of molecules of estradiol dipropionate as of estradiol are absorbed in a given time (175), and there is also evidence that with inunction the hormones and their esters are absorbed at the same rate, molecule for molecule (176).

Some hormones are not absorbed from pellets with sufficient rapidity to be physiologically active and therefore should be injected in oil, even though there is evidence that sesame oil is not always inert (177, 178). Inunction can be used for either local or systemic action (179). The volatile solvents are best for inunction; ether for estrogens, and benzene for androgens and progesterone (180). Only the natural hormones, which do not have to be metabolized, are effective when applied locally at a dose lower than the systemic level (181, 182, 183).

The action of hormones is sufficiently understood to permit clinical administration by the route most convenient for the patient and physician although the necessary level of hormone should usually be established by injection or oral administration. Pellet

implantation is cheap and convenient (184, 185, 186), as is also the injection of aqueous suspensions of hormone crystals which are readily sterilized (187, 188, 189). However, oral administration is still considered the method of choice with the cheaper esterified hormones (190, 191, 192, 193). Sublingual application of both estrogens and androgens has been introduced (194, 195, 196, 197) in an attempt to retain the advantages of oral administration without using appreciably more hormone than by parenteral administration. While inunction (198, 199) is convenient, control of the amount administered is difficult, and the effectiveness varies with the vehicle (200).

Constitutional action.—A nomenclature has been introduced designating as corticoid, leutoid, folliculoid, and testoid, substance having the same activity as the secretions of the adrenal cortex, corpus luteum, ovarian follicle, and testis, respectively (201). It has also been proposed that the suffix 'desmic' be added to the name of the structure stimulated (202). However, there appears to be considerable inertia to the general use of these terms. The discovery that the sex hormones have anesthetic properties led to a study of the anesthetic value of seventy-five steroids and the factors influencing it (203 to 207) but these steroids are not likely soon to be used in practical anesthesia. Renal hypertrophy has been the subject of a recent review in which it was demonstrated that sex hormones alone will not prevent atrophy of the kidneys of hypophysectomized rats. This does not necessarily imply a separate renotropic hormone since thyrotropic and growth hormones are possibly responsible for some of the renotropic effects of the hypophysis (208).

Androgens raise the erythrocyte count and the hemoglobin content of the blood of castrated rats (209) and men (210). Hypophysectomy causes anemia with inactive erythropoiesis (211), but testosterone propionate partially restores the hemoglobin content of the blood and raises the erythrocyte count to high normal by increasing erythropoiesis, while estrogens have the opposite effect (212). A leucopenia associated with normal estrus occurs in the rat (213), and administration of estrogens causes a marked neutrophilia followed by a neutropenia in the dog and monkey (214). In dogs large doses of estrogen also cause an aplastic anemia; a decrease in the number of red blood cells, reticulocytes, and thrombocytes; and the occurrence of granulocytopenia after

an initial leucocytosis (215). Due to its vasodilating property testosterone propionate has been reported to be beneficial in angina pectoris (216). Serum cholestrol varies with the endocrine state of the monkey (217).

Metabolism of Sex Hormones

Inactivation of sex hormones.—The inactivation of estrogens in the body is an intriguing problem. Only a small fraction is excreted in the urine (218), and the liver is quite generally assumed to be the inactivating organ (219) since 98 per cent of the estrogen is destroyed without getting into the general circulation if it is absorbed through the portal system (220). Partial protection to the estrogen is afforded by covering the hydroxyl group. Methyltestosterone is inactivated less rapidly than testosterone (221), and vitamin B complex is necessary for inactivation of estrogen (222). Both of these findings are of interest in relation to the discovery of an estrinase resembling (223), but possibly not the same as, tyrosinase (224). Estrogens have been inactivated by oxidation with laccase (223).

When large quantities of estrone are injected into man only 10 per cent is excreted in the urine (218). After intravenous injection into the dog, only 10 per cent remains in the blood after ten minutes and practically none after twenty-four hours. However, in the same twenty-four hour period 34 per cent to 48 per cent is recovered from the bile and a like amount during the second twenty-four hours; these facts suggest an interhepatic circulation of estrogens and a storage of large amounts for over twenty-four hours, thus throwing doubt on the hypothesis that in intact animals estrogen is rapidly destroyed by the liver (225). On the other hand, Longwell & McKee (226) found only 8 per cent of the estrogen in the bile, which may be due to a difference in experimental procedure. Evacuation of the guinea pig's gall bladder is poor during the second half of pregnancy and in castrated females which have received estrogens and progesterone (227).

As much as 30 per cent of injected stilbestrol has been recovered from the urine of rabbits (228). Excretion of pregnandiol during the nonluteal phase of the cycle seems to be doubtful and earlier results obtained have been suggested as due to faulty methods (229). Androgens inhibit pregnandiol excretion only if the corpora lutea are inhibited (230), and progesterone does not affect the

rate at which estrogen is excreted in the rabbit, which excretes 7 per cent to 11 per cent of injected progesterone as pregnen-3 (α), 20 (α)-diol (231). 5-pregnen-3 (β)-ol-20-one can be converted *in vitro* to dihydroisoandrosterone (232).

Colorimetric estimations of androgens can be made, and by this method androsterone, testosterone, and dehydrosterone can be separated (233, 234). A comparative analysis of the relative amounts of androgen and ketosteroid in the urine may furnish a reliable index of the adrenal cortical hyperfunction associated with virilism (235), except possibly in children (236). Excretion of both estrogens and androgens is reduced in the urine of eunuchoid and castrate men (237) and is raised to normal by injection of testosterone propionate. Ketosteroid excretion during the second and third month of pregnancy has been tested in a small group of women and is reported to be higher when the fetus is a male (238). Excretion of steroids increases in acute vitamin B deficiency (239).

Biological Action of Sex Hormones

Many effective sex hormone substances are available for treatment of menstrual disorders (240), the limiting factor being recognition of the nature of the deficiency (241 to 245). Our knowledge of the endocrine glands does not suffice to explain all the disorders of menstruation and pregnancy (246), but many procedures for control of certain menstrual disorders and menopause have been advanced (247 to 258). With certain endocrine imbalances, thyroid extracts are often more successful than sex hormone therapy (259, 260, 261). It has been emphasized that the sex hormones are fundamentally constitutional drugs irrespective of their effects on the secondary sex apparatus (262), and even moderate doses of estrogen given during the normal cycle suppress corpus luteum function (263). There seems to be considerable variation in the condition of the vaginal epithelium following normal or surgical menopause (264), and the vaginal glycogen content, as demonstrated by the iodine vapor method, seems to be a sensitive index of estrogenic activity (265). Estrogens are more successful in treatment of kraurosis than of leucoplakia (266), but have no effect upon bacteria-free cultures of *Trichomonas vaginalis* (267).

Androgens prevent extreme enlargement of the hypophysis and partially protect against the stunting of growth, the loss of hair, and the atrophy of the skin and sebaceous glands produced in the

rat by estrogen (268). Androgens also prevent hyperossification in mice but synergize the action of estrogen on bone in the pigeon (269). On the other hand, acne resulting from increased amounts of androgen is inhibited by estrogen (270), while estrogens enhance the stimulation by androgen of the combs of 'limited diet' chicks (271). More than 5000 μg. of testosterone is required to nullify 1 μg. of estrone in its effect on the nipple of the guinea pig (272).

Estrogens.—The interest in both the natural and synthetic estrogens is attested by recent reviews (273, 274), but in spite of the extensive use of these substances, the sow's ovary remains the only one in which the estrogens have been identified. The follicular fluid contains α-estradiol, and the total ovary contains about equal amounts of α-estradiol and estrone. Equilenin and a stereoisomer of estrone are the only sex hormones which have been totally synthesized to date (275, 276). Estrogenic hormones have been extracted from testes of several species (277).

Growing follicles are accepted as the source of the estrogen in the ovary, but there still remains some question as to whether the cells of the granulosa or theca or both actually produce the estrogen. A careful study of the thecal gland in the guinea pig points to its being the probable source of estrogen (278), while in the baboon the fact that small amounts of estrogen produce an atrophy of the granulosa cells of growing follicles suggests these cells as the source of estrogen inasmuch as it is known that the presence of sufficient amounts of hormone suppresses the cells which produce it (279). The fact that inhibition of the ovary is reflected by perineal detumescence makes it possible to estimate the amount of estrogen produced during the estrous phase of the cycle in the baboon (280, 282). Zondek & Sklow (281) postulate that a proestrogen is formed in the ovaries during the early stages of stimulation by gonadotropins, which may have some correlation with the finding that although pure follicle-stimulating hormone will induce growth of the follicles, estrogen is not secreted unless some interstitial cell-stimulating hormone is added (20).

One of the most interesting recent findings is that there appears to be a cyclic disappearance of the effects of minimal amounts of estrogen in castrated women (283), monkeys (284), and rats (285), which roughly corresponds to the normal cycle, and it is thought that this cyclic change in threshold is due to the adrenals (286, 287). Responsiveness to estrogen diminishes in mice after a three

weeks' period of continuous administration (288), but in the monkey the uterus is much more responsive to estrogen stimulation immediately after bleeding than when it has remained for some time in the inactive state, as in the castrate animal (289). This condition is similar to the priming phenomenon in rats which, when used as a preliminary step in the assay of estrogen, makes it possible to obtain a very accurate comparison between the unknown estrogen and estrone (290). The variability and unresponsiveness of the castrate guinea pig makes it a poor assay animal (291).

Besides controlling the female reproductive system, estrogen has various other physiological effects. Relatively small doses of estrogen produce a larger rise or a smaller fall in the basal metabolism than large doses (292). Injection of 100 μg. of stilbestrol daily into castrated rats tends to increase the blood sugar levels after twenty days and the liver glycogen after five days; muscle glycogen is not affected (293). The rate of oxygen consumption is also increased with prolonged treatment (294). Estrogens readily induce lipemia in birds, and although in mammals this effect has previously been demonstrated only in rabbits, it is now known that a moderate dose of estradiol benzoate in rats maintained on a diet rich in fat induces a moderate lipemia (295). The inhibition of the testis by estrogen results from suppression of the hypophysis, and recovery is relatively rapid (296, 297) although it may be only partial in some cases (297). Treatment of immature rats with estrogen has been reported to cause the hypophysis to be in a state of hypersecretion amounting to exhaustion (298). Although it is known that suppression of the hypophysis by administered estrogen results in a depression of the gonads and of body growth, it is not possible to separate these two effects (299) even though androgens partially protect against the depression of growth by estrogen. Depression of growth by estrogen is more pronounced in castrates than in normal females (300). Hypertrophy of the hypophysis so readily induced by estrogens in rats and some strains of mice does not occur in guinea pigs (301).

The extensive clinical use of diethylstilbestrol has prompted a very thorough study of this substance in rats (302, 303, 304, 305). It has been found to produce essentially the same effects as the natural estrogens, and without any greater toxic effects (306). The toxic effects of all estrogens are usually proportional to their

estrogenicity (307). Clinical comparisons of these two classes of estrogens (308, 309, 310, 311) agree with the results in animals, and because of the cheapness and convenience of application (312), stilbestrol and its esters (313, 314, 315) are very often the estrogens of choice. They are usually given orally (316, 317, 318) and in an interrupted schedule approximating the estrogen phase of the cycle (318). The nausea and vomiting following administration of diethylstilbestrol is comparable to that of early pregnancy (319). Such patients may be desensitized by starting with a low dose and gradually increasing it since the reaction is directly correlated with the size of the dose (320). In fact, a few cases of refractoriness have been reported (321). Pregnant women can tolerate doses of estrogen many times larger than those which will produce side effects postpartum, this fact indicating a ready detoxification during pregnancy (322).

Castration seems to give the best therapeutic results with carcinoma of the prostate (323), but estrogens are still being used effectively in both hypertrophy (324) and carcinoma of the prostate (325 to 329). The amount of 17-ketosteroids excreted by patients with prostatic carcinoma lies within the normal range, but castration causes a reduction in the amount excreted (330) which is correlated with improvement. Artificial cryptorchidism does not cause regression of the prostate in dogs (331). Estrogen produces a slight increase in the weight of the prostate of castrated rats but large doses will decrease the enlargement due to injected androgens (332). In the human epithelial metaplasia of the prostate is present at birth, undoubtedly due to estrogens from the maternal organism since injection of estrogen following birth causes this metaplasia to continue (333). Androgen does not protect against the squamous metaplasia of the prostatic epithelium in the rat caused by estrogens (334). Male prostates are more responsive to ovarian and adrenal androgens than female prostates (335).

The relation of estrogens to carcinogenesis has recently been reviewed (336, 337, 338). Therefore, it is necessary to state only that while estrogens are apparently carcinogenic with large doses and chronic treatment, their use in therapeutic doses should not be condemned (339). Certain side reactions involving vascular changes have been reported as occurring, especially in the brain of the fowl, following large doses of diethylstilbestrol (340, 341). By the method of intravaginal application of desiccated blood,

which allows accurate tests for estrogen in less than 5 cc. of blood it has been found that the cells contain twice as much estrogen as the plasma in nonpregnant women, but during pregnancy it is equally distributed (342). A new synthetic estrogen 118B [2,4-di (parahydroxy phenyl)-3-ethyl hexane] has recently been introduced (343).

Progesterone.—There seems to be little doubt that the cells of the corpora lutea are the source of progesterone although the fluid in the early corpus luteum of the human contains the same concentration of estrogen as liquor folliculi (344). In the rat the size of the corpus luteum undergoes a cycle corresponding to the estrous cycle (345), and if pseudopregnancy or pregnancy intervenes, activity of the corpora lutea is reflected by their size even during postpartum lactation (346). A persistent functional corpus luteum of eighteen weeks has been described in a nonpregnant woman (347). The interstitial cells of the ovary are formed from the theca of atretic follicles in most species, but are formed from the breakdown of old corpora lutea in certain strains of mice where they also secrete androgen under certain circumstances (348). The androgenic activity is not due to progesterone, either alone or in combination with estrogen, as postulated by Castillo & Trabucco (349). Granulosa cell tumors and thecomas are feminizing while arrhenoblastomas initially possess masculine potentialities (350, 351, 352). A bursting type of atresia exists in the birds allowing the large quantities of yolk to be phagocytized, and the postovulatory follicle of the bird forms nothing comparable to the mammalian corpus luteum (353, 354).

Progesterone, as would be expected, prevents formation of corpora lutea in immature mice and causes involution of corpora lutea in mature mice, without inhibiting follicular growth. Although prolonged treatment with progesterone is reported to inhibit estrus (355) and ovulation is inhibited while the corpora lutea are functioning (356), suppression of androgen production is the only effect of progesterone upon the testis. Spermatogenesis and the size of the testis are not affected (357), indicating that only interstitial cell-stimulating hormone is suppressed. Five milligrams of progesterone daily accelerate growth in immature females, but not in males (358). Although progesterone is usually administered only after priming with estrogen, Hartman & Speert (163) have shown that progesterone alone in adequate doses

will evoke the characteristic reactions of this hormone even after long periods of castrate atrophy of the uterus, but the method of administration is important (359). Progesterone has an apparently specific effect on the stromal nuclei of the endometrium in the mouse (360) which may be used to detect the presence of minute amounts of progesterone in the presence of either estrogen or androgen. A micromethod utilizing ultraviolet absorption spectrophotometry has also been advanced for detecting small amounts of progesterone in serum, but does not distinguish between related steroids (361). Progesterone causes an increase in the amount of basal epithelial fat in the rabbit uterus which is more marked when estrogen is also acting (362). The action of estrogen plus progesterone is spoken of as both synergistic and antagonistic (363), but these terms probably have little meaning in regard to uterine and vaginal physiology since both hormones acting together produce a new type of histological change (364 ,164).

The andromimitic activity of progesterone and the ability of progesterone to alleviate adrenal insufficiency under various conditions (365) has led to further study of the androgenicity of both progesterone and desoxycorticosterone (366, 367, 368). Neither desoxycorticosterone acetate nor progesterone has an androgenic effect on the sparrow bill (369) nor does the androgenic activity of the adrenals seem to be due to desoxycorticosterone.

While the therapeutic value of injected progesterone and orally administered pregneninolone is not conclusive (370), these substances elicit uterine responses (371) and are fairly effectively used in dysmenorrhea, functional bleeding (372, 373, 374), and threatened abortions (375, 376, 377). However, most abortions occur at about the time of the second or third missed menses (375) and with either high or low levels of urinary pregnandiol (378).

Androgens.—The substance secreted by the testis is assumed to be testosterone although it has been identified only in the bull testis (379). From studies based on mammals it seems to be accepted that the interstitial cells are the sole source of the androgens produced by the testis, and this opinion has been confirmed by histochemical study (380). On the other hand, careful study of the testes of many species of birds has failed to reveal any correlation between histologic or cytologic characteristics of the intertubular elements and relative masculinization. A much closer correlation

exists between spermatogenic activity and androgen production. However, true interstitial cells of Leydig can be stimulated to appear in the sparrow and male hormone production obtained, without spermatogenic activity, by suitable administration of gonadotropins (381), but since these cells are lacking in the normal sparrow testis androgen must be assumed to be produced under either condition.

Administration of androgens, as well as of estrogens, may modify embryonic development of the undifferentiated gonads of some species of fish (382), amphibia (383, 384), and reptiles and birds (385), but it is doubtful that these agents are involved in the normal control of embryonic sex development (386). In mammals these hormones appear to be without evident permanent effect on either undifferentiated or differentiated gonads (387, 388, 389, 390), but they readily induce masculinization or feminization. In the rat fetus androgens antagonize estrogens in the male, and estrogens antagonize androgens in the female (388), but contrary to the situation in the adult, androgens are much more potent than estrogens in milliequivalents (388). Androgens activate the ovaries of lizards (391) and sparrows (392) during the nonbreeding season, presumably through the hypophysis, and in the castrated female lizard directly stimulate the accessories (391). Androgens also readily masculinize regenerating anal fins in certain adult female fishes (395, 396, 397). Testosterone inhibits gonadotropic activity of the hypophysis in rats although this effect may be limited to luteinizing hormone production (393). It also will produce functional development of just formed corpora lutea, possibly by evoking release of prolactin (394).

The role of androgens in maintaining spermatogenesis in the hypophysectomized mammal is not well understood (398). Since doses larger than those which suppress the hypophysis are required for maintenance of spermatogenesis, it seems improbable that spermatogenesis is maintained by endogenous androgen unless it is more effective near its source (399), for which there is as yet no evidence (400, 401). The ground squirrel testis appears to be more highly responsive to androgen (402, 403, 404) than the rat testis since spermatogenesis is not only maintained but also is stimulated (405) with appreciably less androgen, a condition which may be associated with the fact that the ground squirrel is a seasonal breeder. Androgens have not so far been found to maintain

spermatogenesis in man (406) and are presumably not very effective in this respect in the monkey. However, in the hypophysectomized monkey androgens inhibit regression of the testes to a greater extent than do gonadotropins (120).

Colchicine mitoses have been used to detect very slight stimulation of male accessories by androgen. Mitosis in the seminal vesicle, but not in the prostate, may also be induced by 100 r.u. of estradiol benzoate, while 80 mg. of progesterone causes hypertrophy of the glandular epithelium and connective tissue of the accessories, with mitoses most numerous in the ventral prostate (407). The anterior prostate in the guinea pig is least affected by castration (408). In immature rats, when organ weights are used as criteria, the uterus is more responsive to testosterone propionate than are the prostate and seminal vesicles (409). In intact female rats the response to androgen depends on the stage of the cycle, the response during diestrus being the same as in the spayed animal (410). Prolonged treatment with androgens decreases glucose tolerance in rabbits and humans (411, 412).

Surprisingly enough, the clinical use of male hormone in men has been somewhat disappointing and has found its greatest use in the disorders of women (413) although it is still used along with chorionic gonadotropins in treatment of cryptorchidism (414). The therapeutic value of androgens in gynecology, recently reviewed by Geist & Salmon (415), seems to stem from their ability to nullify or modify the action of estrogens, to inhibit the gonadotropic activity of the hypophysis and thus suppress or decrease the production of estrogens by the ovary, to inhibit the proliferative process in the endometrium, and to inhibit the reactivity of uterine musculature. Androgens are effective in functional menometrorrhagia, functional dysmenorrhea, premenstrual tension, and mastopathie, and postpartum engorgement of the breasts; and in certain types of menopausal syndrome they are effective either alone or in combination with estrogen (416). Nausea of pregnancy is relieved by androgen if blood estrogen levels are high (417). Androgens are also effective in alleviating nocturia due to hormonal imbalance (418) and have been used following surgical removal of mammary cancer to inhibit possible metastases (419).

The effectiveness of androgens and their esters, using molecular equivalents, varies with the vehicle and mode of application (420).

Oral administration of methyltestosterone and implantation of pellets of testosterone and testosterone propionate are the most convenient methods of application (421 to 425). There seem to be several objections to sublingual applications. The ready increase in sexual characters, vigor, and stamina in the race horse following administration of androgen (426) corresponds to the findings on the effects of androgen on the castrate and eunuchoid man. However, the presence of testicular tissue is reported to modify the effectiveness of the androgens in the eunuchoid patient (427). Androgens are incitants of acne (428, 270) and are essential for the expression of baldness (429). The vague male climactric may be alleviated by the administration of androgen (430), and the relation of sex hormones to growth allows the use of androgen in controlling certain cases of abnormal growth (431). An interesting speculation has been presented that in the brain there is a center in the region of the mammillary nuclei which is responsible for genital development (432).

Control of Mammary Gland Development and Lactation

Mammary growth.—According to the latest theory concerning hormonal control of mammary growth the estrogen produced by the ovary under pituitary gonadotropic stimulation has a twofold action (433). It acts on the hypophysis to increase the output of mammogenic (duct growth) factor and acts secondarily to sensitize the mammary gland to the pituitary mammogen. The latter effect would explain the local action of estrogen when applied to the breast (434). However, some slight mammary growth has been observed in hypophysectomized mice receiving only estrogens (435). It was assumed that the cyclic nature of duct growth was correlated with the increased amounts of estrogen present during estrus, the luteal phase of the normal cycle being too transitory to exert much effect (433). After conception the progesterone, probably acting synergistically with estrogen, stimulates the anterior hypophysis to an increased secretion of mammogen (lobuloalveolar factor), while presumably the direct action of estrogen on the stroma of the mammary gland and on the nipple continues. These features would be sufficient to explain the growth of the mammary gland during pregnancy (433).

The existence of the duct growth factor as a separate fat-soluble

pituitary hormone has been both denied (436) and reaffirmed (437) on the basis of experiments on castrated and hypophysectomized rats. The nature of the sensitization of the mammary stroma by estrogen also remains rather obscure since the idea that it causes a local subcutaneous hyperemia (433) seems to be untenable in view of the failure of several rubefacients to cause mammary gland growth (438). However, the sex hormones seem to be essential regardless of their mode of action. In hypophysectomized male mice, but not in rats (439), slight mammary development occurs with estrogens alone, but if estrogens or androgens are combined with prolactin or growth hormone, lobuloalveolar growth occurs (440, 441, 442). In hypophysectomized rats the results are not clear-cut since Leonard & Reese (439) reported that estradiol dipropionate, testosterone propionate, and desoxycorticosterone acetate, either alone or in combination, gave negative results, while a combination of desoxycorticosterone acetate and estradiol benzoate has been reported to be effective (443). It has also been suggested that under the influence of gonadotropic hormone the ovaries of hypophysectomized rats secrete substances which directly stimulate the mammary glands (444). In intact but not in castrated male mice, feeding desiccated thyroid causes development of the mammary gland (445), and thyroxine in optimum doses increases significantly lobuloalveolar growth with a minimum of estrogen-progesterone (446).

It is evident that interaction of several hormones is essential for optimum growth of the mammary gland. In the male rabbit where the best duct growth following administration of estrogen alone is produced by 120 I.U. of estrone (448), the optimum dose of progesterone in combination with this amount of estrone is 1 mg., but this does not give a maximum response; and 4 to 8 mg. of progesterone added to 120 I.U. of estrone has an inhibiting effect (447). However, by keeping the optimum dose of progesterone constant and varying the amount of estrone, it was found that the optimum level of estrone with this amount of progesterone was between 240 and 960 I.U. This combination of estrogen and progesterone produced mammary glands which, except for the presence of incomplete lobules at the periphery, were indistinguishable from the normal maximal preprolactational proliferation (448).

Although progesterone is more effective in stimulating lobuloalveolar growth in mice when combined with estrogen, the effec-

tiveness of progesterone alone is twice that of pregnenoline, three times that of desoxycorticosterone acetate and dehydroandrosterone, four times that of diethylstilbestrol, sixteen times that of acetoxypregnenoline, and twenty-five times that of methyltestosterone. Testosterone and testosterone propionate in daily doses up to 10 mg. did not induce lobuloalveolar growth (449). In male guinea pigs any dose of stilbestrol which produced distension of the duct also induced lobule development (450).

In evaluating results it is essential to consider the species, the sex, and whether the animal is castrated or hypophysectomized. Such factors as environmental temperature (451) and inanition (452) also affect the mammary glands, but they do not account for all the effects of hypophysectomy. The possibility has not been eliminated, however, that favorable activity of certain preparations or combinations in hypophysectomized animals may be, at least partially, due to alleviation of the symptoms of hypophysectomy which secondarily interfere with mammary development.

Lactation.—The mammary glands are well developed during the period of pseudopregnancy or its counterpart in pregnancy, but lactation does not occur until parturition, necessitating some mechanism to bring about lactation at the proper time. Lactogenic hormone, which induces the fully developed gland to lactate, is present at no higher level in the hypophysis or blood stream of pregnant animals than of nonpregnant animals (453), except in mice (454), notwithstanding the presence of large amounts of estrogen which should increase the amount of lactogenic hormone in both the hypophysis (455, 456) and the blood (457). The low level of lactogenic hormone is thought to be due to the presence of progesterone which, although it causes a very slight increase in the lactogen content of the rat hypophysis (458) but not of the rabbit hypophysis (458), may in suitable combination with estrone suppress or override the action of estrogen (459). Following parturition the precipitous increase in the lactogenic hormone content of the pituitaries which occurs in rats, guinea pigs, and rabbits two to five days postpartum (460) could be accounted for by the presence of estrogen uninhibited by progesterone. The hypophysis, while not necessary during the latter half of pregnancy in mice or for the onset of the secretion of milk (461), is essential for lactation and continued milk secretion, as are also such factors as milk precursor substances, thyroid hormones, and nipple stimulation or

milk removal (462, 463). Total fat and milk fat are both influenced by anterior pituitary extract (464).

In the human there seems to be little clear-cut evidence that lactogenic hormone can be used effectively to increase lactation, which may be due to the fact that in most cases of failure of lactation it is not known whether lactogenic hormone is deficient. A probable relationship exists between the level of milk secretion and the amount of lactogenic hormone in the urine (465) which may prove to be of diagnostic value in determining whether hypogalactia is due to an insufficiency of lactogenic hormone.

In cattle and goats estrogen (diethylstilbestrol) alone will induce development of the mammary glands and cause copious milk secretion. Testosterone propionate synergizes the action of estrogen and is more effective than progesterone (466). In the goat the artificial lactation provoked by the administration of estrogen in combination with crude pituitary extracts is better than that produced by pure prolactin in cattle (468) and rivals that following parturition (467). Progesterone does not seem to be necessary for complete mammary development and lactation in cattle (469).

When administered in sufficiently high doses, the sex hormones inhibit lactation, but the mechanism is not well understood. It may be a suppression of the secretion of anterior pituitary hormones other than lactogen (456) inasmuch as the yield is probably influenced by the availability of the milk precursors in the blood more than by the capacity of the mammary epithelium to secrete milk (456). It has been suggested that the inhibition of lactation by estrogen and gonadotropic hormones depends on the production of nonestrogenic substances by the ovary (470). Clinically both estrogens (471 to 475) and androgens (476 to 480) can be used effectively for relief of breast discomfort in the puerperium. Under conditions which give adequate relief, lactation is not permanently affected if nursing is continued. Neither estrogen nor progesterone affects the regression of the mammary gland of the mouse after removal of young at birth (481).

The impairment of lactation by adrenalectomy can be partially alleviated by all agents known to relieve adrenal insufficiency, and compound E is a highly effective supplement to pituitary lactogen for the initiation of lactation (482). The presence of lactogenic hormone in the adrenals has been reported (483) but has recently been denied (484). Lactogenic hormone has been suc-

cessfully used to control bleeding in menorrhagia-metrorrhagia (485). It is antigonadal in effect, but it is transitory and harmless. Hysterectomy was found to have no effect on the mammary glands of the monkey (486), and further evidence has been obtained that uterine distention does not affect lactation (487). Evidence has also been presented that the mammary glands have both parasympathetic and sympathetic innervation (488).

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DEPARTMENT OF ANATOMY
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PHYSIOLOGICAL PSYCHOLOGY

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PART I. THE FUNCTIONAL PSYCHOSES

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The physiological psychology of today reflects a fundamental shift in emphasis that has occurred in general psychology, away from the older introspections concerning sensations, images, and ideas, and toward more objective and verifiable problems of action, i.e., of the organism's performance in relation to changes in the internal and external environment. Two of the most active fields within physiological psychology are those of the major behavior disorders (1) and of the central nervous system functions. This part of the review deals with those major disorders for which consistent and dependable structural lesions or physiopathological changes have not yet been demonstrated, viz., the schizophrenias, the depressions, mania, and paranoid or paranoic reactions. Following a practice common in clinical medicine, these disturbances are usually designated as functional (2).

Most of the current physiological and biochemical studies in psychiatry focus upon the relationship between the functional psychoses and biological changes that have been observed to precede, accompany, or follow the psychotic behavior. Although this general problem has held the spotlight for more than a century it is still essentially unsolved; but because the demonstration of verifiable correlations between such psychotic behavior and physiological or biochemical changes would greatly simplify both research and therapy in this important field, it still occupies a central position. Whitehorn (3, 4) reports a general trend away from developmental and anamnestic methods in this field and toward cross-sectional physiological studies. Gildea (5) makes a similar case for biochemistry. Alexander (6) presents data to indicate the opposite trend, i.e., toward genetic longitudinal studies and away from the study of cross-sectional physiological states. From the neurologist's point of view, Putnam (7) regards the prospect of solving any but the simplest psychological problems by physiological means as "far distant."

Gildea (8), citing the analytic reviews of biochemical investigations in schizophrenias, depressions, and mania by McFarland & Goldstein (9, 10), concludes that they present a confusing array of conflicting reports suggesting that little contribution toward an understanding of functional psychoses has been achieved by biochemical methods. Gildea interprets many of the negative results, in which correlations cannot be established between psychotic developments and biochemistry, as evidence of something basically abnormal about the biology of the psychotic person and not simply as evidence of a lack of any necessary relationship. Kopeloff (11), in an extensive review of the literature, finds no reliable evidence for an etiological connection between the functional psychoses on the one hand and focal infection, autointoxication, or bacillemia on the other. He concludes that thus far no substantial difference has been demonstrated between the body fluids of normal and of psychotic persons.

Notwithstanding the disadvantages for scientific investigation that it entails, a purely metaphysical mind-body dualism is still the most widely accepted working hypothesis among research workers in this field. The dedication of a scientific journal with a monograph series to this dualism (12) and the devotion of a national research symposium to the "interrelationship of mind and body" (13) indicate the current importance ascribed to this theoretical approach by investigators. Two variants of the "psychosomatic" hypothesis are prominent in the literature of today: (*a*) one assumes that body is fundamental and disorders of mind only the echoes of undiscovered structural or chemical disturbances; (*b*) the other considers mind to be fundamental and bodily disturbances largely the expressions of psychic conflict. Both attitudes are very old (14). Grinker (15) tries to avoid the mind-body difficulty by using central nervous system terms interchangeably with terms taken from psychic theories of the "unconscious" as, for example, cerebral cortex with "ego" and hypothalamus with "id."

A more objective and operational approach, finding increasing application in this field, discards mind-body or psychosomatic dualism entirely. This approach distinguishes only (*a*) between activities of simpler and more complex degrees, and (*b*) between activities that involve interactions chiefly within the organism and those involving interactions between one organism and others. The functional psychoses, accordingly, become disorders in the behavior of the whole organism, comparable in character, origins,

and consequences to functional cardiac disorders, and like them always involving biological activity (2). The advantages of this theoretical development over the older ones are that it does not compel experimentalists to concern themselves with the purely metaphysical "mind-body problem," and that it does not place the origins of organismic action in an "unconscious mind" that is by hypothesis inaccessible (14).

Toxins.—Because of the assumption by such leading clinicians as Kraepelin, Bleuler, and Freud that endogenic or exogenic toxins are at the basis of schizophrenia, considerable interest attaches to investigations in that direction. The review of such work by Kopeloff with its negative findings has been mentioned (11). More recently Stockings (16) has confirmed earlier work pointing to clinical similarities between schizophrenic phenomena and those deliberately induced through mescaline intoxication; he concluded on the basis of these similarities that schizophrenia must result from the action of some mescaline-like toxic amine. Scouras (17) described a catatonic-like syndrome in chronic *Cannabis indica* addiction and suggested that schizophrenic catatonia may result from the action of some exogenous toxin upon the diencephalon.

Reiter (18) could not induce toxic effects in mice through injection of the cerebrospinal fluid of schizophrenic patients. He did obtain temporary remissions in two of four schizophrenic patients through large-scale blood transfusions from normal donors and regarded this as supporting evidence for the toxic origin of schizophrenia; but such remissions follow a great variety of physiologically unrelated and much less dramatic incidents in schizophrenia; and they often occur in the absence of any deliberate interference (so-called "spontaneous remissions"). Den Hartog Jager (19), investigating the possibility of histamine intoxication as a basis for metabolic disturbances in schizophrenia, was unable to find significant differences between the histamine content of the blood of schizophrenics and that of normals. Using a new and very sensitive method of estimating blood amines, Richter & Lee (20) found in depressed, manic, schizophrenic, and paranoic patients less than eight parts per million of β-phenylethylamine or of higher aliphatic amines formed by putrefactive bacteria. They report a reliably increased amino lipid fraction but attribute it to nutritional conditions. They conclude that for the functional psychoses the toxic amine hypothesis ought no longer to be looked upon as anything more than speculation.

Hepatic function.—The search for toxic processes in these psychoses has naturally led to investigations of hepatic function. Quastel & Wales (21, 22), studying detoxication of benzoic acid in catatonic schizophrenics, obtained values below the average normal figures and concluded that catatonia involves liver dysfunction. Ström-Olsen *et al.* (23) were able to find decreased hippuric acid excretion in only five of twenty-eight catatonic schizophrenics and in six of thirty-four schizophrenics without catatonic symptoms. Davies & Hughes (24) reported diminished hippuric acid excretion in fifteen of seventeen catatonic schizophrenics and in forty of seventy-five other psychiatric patients without catatonic symptoms; they conclude that this result is not specific for catatonic syndromes. Berkenau (25), on the basis of similar results, postulates liver damage from toxins and toxic amines as underlying catatonic syndromes in schizophrenia and depression. Gökay & Polvan (26) interpret santonin excretion findings in three depressed persons as pointed to a necessary relation between hepatic insufficiency and melancholia; two normal persons constitute the controls.

De Jong (27) produced catatonia-like syndromes in sixteen cats by ligation of the hepatic artery and in two dogs by anastamosing the inferior vena cava and the portal vein, thus partially shunting out the liver. Lundquist (28), in a study of blood protein and quinine-resisting lipase, found no evidence of hepatic function disturbance in eighteen schizophrenic and depressive patients.

Nitrogen balance.—Gjessing (29, 30) has reported alternating phases of nitrogen retention and excessive nitrogen excretion that correspond with alternating periods of excitement and stupor in catatonic patients. He has set these patients apart as cases of "periodic catatonia." He believes that some toxic substance, a biogenic amine or a product of protein metabolism, is formed at the time of a change in nitrogen balance and that this acts directly upon the diencephalon to produce the catatonic syndrome. Thyroid administration, by increasing nitrogen elimination, is reported to relieve the catatonic attack. Stokes (31), in an exhaustive review of physiological research in periodic catatonia, considers Gjessing's results to be unequivocal and exceedingly promising. Gildea (8) points out that with the development of micromethods for amino acid determinations further advances along the lines indicated by Gjessing are to be expected. On the other hand, Watterson (32) could find nothing specific about the symptomatology of the patients set aside as periodic catatonics when he visited Gjessing's

clinic. To him their clinical characteristics seemed very variable and did not constitute a distinct syndrome. Hemphill's recent critical review (33) raises doubt as to the validity of Gjessing's criteria and conclusions.

Cardiovascular changes.—Freeman (34, 35), using the cyanide method, reported mean arm to carotid circulation time consistently increased for schizophrenics; but Finesinger *et al.* (36) failed to corroborate this finding. Gottlieb (37) also failed to obtain Freeman's results although following his method closely; his schizophrenics showed no significant difference from normal controls. Gottlieb found both depressed and manic patients to have increased mean circulation time that was suggestive of being significantly different from the normals. Mean values for manic were within 0.01 sec. of those for retarded depressed patients. No correlation between rate of blood flow, on the one hand, and type, duration, severity, or outcome of schizophrenia, on the other, was found by Rosenbaum *et al.* (38), nor was total volume of the brain reduced. Their schizophrenic patients showed no significant differences in rate of blood flow from normals; but a tendency toward greater variation was present in the schizophrenic group.

Olkon (39) has described capillary abnormalities in the dorsum of the hands of a large group of schizophrenics under twenty-five years of age; these included sparseness, reduction in color intensity and in comma-like structures, increase in exceptional units and in tortuosity, and progressive development of stellate, spiral, and crescent forms. He reported that the severity of the clinical disorder corresponded with the degree of capillary derangement. The rate of capillary blood flow was more variable than in normals. He regards these results as indicating that schizophrenia is fundamentally a vegetative and metabolic disorder. Cotton, Lewis & Egenhofer (40) established no correlation between capacity of the retinal vascular bed and either schizophrenic or manic-depressive syndromes. They suggest that such measurements have possibilities as prognostic criteria since more cases having larger vascular beds improved. The differences, however, are small. Their data show four unimproved or deteriorated among the lower nine cases and three among the upper, arranged in ascending order of retinal vascular bed capacities; and the total number of cases, eighteen, is too small to be significant in the presence of so wide a range of values.

Freeman (41, 42) found that under basal conditions and immediately after exposure in the nude to 30°C. at 20 per cent

humidity, schizophrenics had warmer extremities than normal controls but showed more rapid cooling at 24°, 20°, and 15°C. Their reactions at 44°C. were similar to those of normals, as also were rectal temperatures, and skin temperatures of trunk, head, and proximal areas on extremities, under all conditions. Freeman concluded that peripheral vasodilatation was not impaired while constriction was; this contradicts earlier reports (43) that reactive hyperemia is much impaired or absent in schizophrenia. The evaporation of insensible perspiration under basal conditions fell steadily in the schizophrenic group but somewhat less than in the normal controls; and the rate of oxygen consumption was slightly lower although the observed greater restlessness of the normals may account for this difference (42). Responses to the general metabolic stimulation of dinitrophenol were definitely less in the schizophrenic group. In another study, Freeman (44) compared the skin temperatures of ten normals and ten chronic schizophrenics before and during induction of deep cyclopropane anesthesia. No significant differences appeared in rate or rapidity of temperature rise or in diversity in the responses of the lower extremities. Freeman concluded that the neurogenic mechanisms in schizophrenia are capable of evoking normal vasodilatation under conditions of cyclopropane anesthesia. Abramson *et al.* (45), using the venous occlusion plethysmographic technique, found that diminished blood flow in the hands of schizophrenics could be increased by local application of heat to the same values as those obtained for normals by heating. They ascribed the initial reduced flow to excessive vasoconstriction and not to defects in the peripheral vascular tree. Montgomery has pointed up the same interrelationship in normal physiology and has discussed its implications for experimentation with vasodilator and vasoconstrictor drugs (46). In view of the complexity of central and peripheral mechanisms involved in vasodilatation (46, 47, 48), speculation as to the meaning of vasomotor changes in the functional psychoses on the basis of the foregoing results would be premature.

Oxidative processes, etc.—Rheingold (49) found a low oxygen consumption rate in schizophrenia and attributed it to a disturbance of mechanisms regulating cell respiration and to occult thyroid hypofunction which did not, however, respond to thyroid therapy. Earlier claims that the immediate neurophysiological effects of insulin and of metrazole administration could be attributed to cerebral anoxia (50, 51, 52) are not supported by the

work of Fraser & Reitman (53) who induced anoxia through nitrogen inhalation, with provision for removal of the expired carbon dioxide; the neurophysiological manifestations did not follow the insulin or metrazol patterns. Levine & Schilder (54) and Green & Adriani (55) in general corroborate Fraser & Reitman's report. Green & Adriani find no comparable after-effects even after nitrogen inhalation up to fifteen minutes.

Vestibular function.—The nystagmic response to caloric stimulation and to rotation is significantly lower in schizophrenics than in normal controls, according to Angyal & Blackman (56), and lowest in the most apathetic and generally unreactive. Fifteen minutes after alcohol ingestion schizophrenics showed an increase in nystagmic beats to caloric stimulation followed by a steady decrease in rate at thirty and sixty minutes, whereas normals showed an initial drop at the fifteen minute interval followed by a rise (57). Angyal & Blackman call this a paradoxical reaction; but this reviewer notes that, starting from a low pre-alcohol rate, the schizophrenic record rises to almost exactly the same point as that reached at the lowest point in the normal drop. In the schizophrenics it then falls away again as the alcohol diminishes, while in the normals it rises again. In other words, the alcohol seems to extinguish temporarily the differences between the two groups, which return to their former level, up or down, as the alcohol effect wears off. The schizophrenics showed no significant change in nystagmic reaction to caloric stimulation under increased carbon dioxide tension.

The turning movements of blindfolded subjects, following caloric stimulation of the ear with water at 25° and 28°C., were significantly lower in a group of twenty randomly selected schizophrenics than in twenty normals, as measured by the degree of rotation about the vertical axis of the body. This difference obtained for the mean total reaction, for the degree of reaction per unit time, and for the total duration of the reaction. Angyal & Sherman (58) interpret their data to indicate either reduced reactivity of receptors or conductors or decreased general muscle tonus. Freeman & Rodnick (59) studied the station of normals and schizophrenics following rotation in a revolving chair. Recording was begun after the subject could stand unaided. Within the first ten seconds of recording the schizophrenics showed significantly less difference in stability before and after rotation than the normals; at twenty seconds the differences were not significant; at

thirty seconds the records of both groups were similar. They suggest that either there is a different vestibular and cortical response to postural stress, or the postural substrate in the two groups is different.

Respiration.—Respiratory patterns studied by means of thoracic and abdominal pneumographs in schizophrenics and in normal controls, both instructed to daydream, revealed somewhat prolonged periods of apnea following each expiration in both groups, but of more frequent occurrence among schizophrenics (60, 61). Respiratory patterns characterized by a smaller volume of tidal air and greater regularity were found by Thompson & Corwin (62) more often in a group of twenty-five hospitalized schizophrenics than in normal college students.

Miscellaneous.—Edema of the extremities in the absence of demonstrable systemic disease is a common finding among inactive schizophrenics that has had a variety of interpretations. Williams (63), working with normal subjects, obtained marked postural edema when the upper extremity was held continuously in a dependent position for four and one-half hours, the average volume increase in the hands being 18.3 per cent and in hand and forearm together 10.3 per cent. Looney (64) reported relative muscular inefficiency in schizophrenics as compared with normals living in the same environment; the same rate of lactic acid disappearance in both groups suggested good response of carbon dioxide mechanisms under stress of exercise. The effects of chronic relative inactivity in the patients were not ruled out. Sherman (65), in a study of optokinetic nystagmus, found no significant differences in the ratio of nystagmic jerks to the number of passive visual stimuli as between normals and schizophrenics. He attributes differences earlier reported (66) to the inability of some patients to cooperate so that they were not actually receiving the stimulation.

Freeman & Rodnick (67) studied the effects upon schizophrenics of an atmosphere of oxygen saturated with water vapor at 41°C., which prevented heat loss from the lungs. Responses in blood pressure, heart rate, and respiratory rate, and amplitude were definitely less than those of normal controls. They interpret this as indicating reduced adaptive autonomic reactivity. Claims that schizophrenic emotional disorders, as opposed to manic and depressive, are correlated with structural differences in the thalamus received no support from biometric analyses of thalami from these groups (68), which showed no significant differences. Milhorat,

Small & Diethelm (69) found no correlation between degree of leukocytosis and any of the disorders according to psychiatric classification; but there was some correlation between the degree of leukocytosis and intensity of pathological emotion, regardless of direction and of diagnosis.

Contradicting the report of Yorshis & Gottlieb (70), no significant deviation from normal blood-group distribution was found in 257 manic depressive and 67 schizophrenic patients by Thomas & Hewitt (71). They found no difference between 200 normals and 300 psychotics in isoagglutinin titres (72); and the combined totals represent the frequency of the agglutinin titres among 500 of the general population. Horvath & Corwin (73) reported excretion of creatine in 86 per cent of urine specimens from adult schizophrenic males even on a meat-free diet, as well as from all females. Hyde (74) in a study of normals found that ability to transform exogenous creatine into creatinine varied irrespective of age, sex, muscular development, and of degree of creatinuria on a creatine-free diet. He found no correlation between capacity to retain exogenous creatine and the extent to which retained creatine was dehydrated or degree of creatinuria on creatine-free diet. Decreased rate of parotid secretion has been found by Strongin & Hinsie (75) to be a reliable early means for differentiating between various forms of depression.

In electroencephalographic studies, Lemere (76) concluded on the basis of strength of the alpha rhythm that electric cortical energy production is low in schizophrenia, toxic and organic psychoses, and high in affective reactions, including manic depressive and many paranoid states. His data, however, show a considerable range of values, e.g., 22 per cent catatonic schizophrenics having "strong" and 36 per cent depressed having "weak" alpha rhythm. He postulates a low energy cortex, secondary to primary diencephalic inadequacy, as the origin of schizophrenia and an overactive diencephalon as the origin of mania and depressions, and advocates therapeutic attempts to regulate diencephalic output in functional psychoses. Hecker (77) considers schizophrenia to be due to debility and depletion of functional potential in cortical neurons. Finley & Campbell (78), however, found paucity of alpha waves in a very small percentage of 500 schizophrenics. They distinguished fifteen different EEG patterns in their schizophrenic group, no one of which included more than 13 per cent of the total; and nothing appeared that could not be

duplicated in agitated or retarded depressions, mania, and neurosyphilitics. Jasper (79) and Knott (80) have reviewed electroencephalography in relation to functional psychoses.

The literature on chemical and electrical "shock therapy" is too extensive and contradictory to be reviewed here. An increasing number of reports, in which adequate control groups have been included, claim equally successful results as to outcome in the functional psychoses when nonspecific psychiatric therapy is competently employed with adequate facilities and time. It is believed by some workers that "shock therapy" operates through mechanisms similar to those in serious accidents and acute intercurrent infections, which have long been known to influence markedly the course of functional psychoses and particularly of schizophrenia (81 to 96).

In a review of clinical and experimental studies on the biology of mania and depression, Cameron (97) was unable to find significant differences that would support the current hypothesis that these are physiologically and biochemically opposed phases of a single disease entity. Nagel (98) has reviewed recent European physiological work on schizophrenia, some of which could not be included above.

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PHYSIOLOGICAL PSYCHOLOGY

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PART II. PHYSIOLOGICAL CORRELATES OF BEHAVIOR

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The material in this section is in a large part limited to the effect of lesions in the nervous system upon the behavior of organisms, with emphasis upon the complex types of behavioral change commonly described as learning, reasoning, and "higher mental processes." This material has been covered up to 1940 by Marquis (1). The same author (2) has recently presented a more general and complete discussion of the status of the neurology of learning. The nature of cerebral organization is described and the techniques for studying cerebral localization are explained. The effects of lesions in the occipital, temporal, and frontal areas are presented and interpretations advanced relating the described deficits to the position of the various animal subjects in the phyletic series. The mechanisms that may operate in recovery from cerebral lesions are critically discussed. Concerning the nature of neural modifications Marquis points out that "there is at present no satisfactory theory of the nature of the relatively permanent synaptic changes which would seem to be necessary to account for learning." An introductory text on physiological psychology has been presented by Hathaway (3). Two thirds of the book are devoted to a description of basic neural anatomy and neural physiology and one third to a discussion of physiological mechanisms underlying emotion, language, thought, and motivation. To facilitate reading by the elementary student, references to 90 per cent of the cited experiments are omitted. Goldstein (4) has recently written a book on the effects of brain injury in war. The general, neurological, and mental symptoms of brain injuries are discussed and their physiological and behavioral significance evaluated. Considerable emphasis is given to psychological examination and techniques of therapy.

Basic behavior patterns, innate organization; motor skills.—

Destruction of the anterior third of the cerebral cortex results in slight but statistically significant increase in the running activity of guinea pigs as measured by the revolving cage technique. Furthermore, a fairly high correlation between mean preoperative and postoperative activity was found by Smith, Peterson & Rayson (5). In similar tests on the rat, Beach (6) reports that subjects with the posterior half of the cortex destroyed showed a mean increase in activity of 27 per cent, whereas destruction of the anterior half of the cortex caused a mean increase of 122 per cent. Animals sustaining injury greater than the average of the group showed less marked increases in postoperative activity. The greatest increases in activity were found in animals with less than 30 per cent of the cortex destroyed. Lesions in the corpus striatum had no consistent effect on activity (7). Testing by means of a pneumographic recording cage, Kennard, Spencer & Fountain (8) found a marked increase in activity in monkeys associated with frontal lesions. An equivalent degree of restlessness was judged to appear in only 6 per cent of normal monkeys. Hyperactivity was most marked following bilateral destruction of areas 8 to 12, but was still marked when only areas 9 to 12 were ablated. The observed hyperactivity was clearly affected by visual stimuli. Involvement of the caudate head or of the putamen produced no observed defects.

Considerable data bearing on the hormonal and neural factors related to the time, vigor, and patterning of the copulatory responses in the male rat have recently appeared. Stone (9) finds that male rats kept at a constant weight from thirty to fifty days of age show a mean retardation of ten days in initial appearance of copulation. Daily injections of 0.25 mg. of testosterone propionate reduce this retardation to 3.3 days. The effect of cortical lesions upon sexually active male rats has been described by Beach (10). Lesions up to 19 per cent of the total cortex did not abolish copulatory behavior in any male. Lesions from 20 to 75 per cent eliminated copulatory responses during some tests for many of the males. With lesions involving 39 to 75 per cent of the cortex, all six males failed to copulate. Injections of testosterone propionate into postoperatively sexually nonactive rats frequently reinduced copulatory behavior. These data indicate that cortical lesions reduce the responsiveness of the male rat and thus decrease the ease of arousal of the sex act without affecting the behavior pattern

involved. Similar data were obtained by Beach (11) on the effect of partial decortication upon the copulatory behavior of rats raised in isolation. The proportion of copulators was inversely related to lesion size, but the average number of copulations in the successful tests was not decreased nor was the pattern of the response seriously affected. The greater severity of the effect of lesions in the latter experiment—no animal with a lesion greater than 30 per cent copulated—may be explained by differences in previous experience of the subjects, in the nature of the test sessions, and the lack of postoperative testosterone injections. The latter suggestion finds experimental support in a later publication (12) showing that testosterone propionate increases the susceptibility of the inexperienced male to sexual excitement and widens the range of stimuli capable of eliciting copulatory responses. In an analysis of the adequate stimuli for mating behavior, Beach (13) has demonstrated that destruction of the eyes, the olfactory bulb, or the trigeminal nerve reduces copulatory behavior in the inexperienced rat; and combined inactivation of any two of these peripheral mechanisms abolishes such behavior. Sexually experienced males cease to copulate only after destruction of all three sensory systems described. A detailed critical review of the central nervous mechanisms involved in reproductive behavior of vertebrates has recently been presented by Beach (14).

The effects of partial and complete section of the corpus callosum on laterality in sixteen patients were studied by Smith & Akelaitis (15) using a battery of 97 measures. No obvious effects on laterality and motor organization were found. Unilateral disturbances of motility appeared in only three cases, all characterized by other organic involvements besides callosal section. Temporary disturbances in laterality were found in left-sided and ambidextrous patients in performances involving bimanual eye-hand coordinations and unimanual tool using and manipulations. Eyedness and writing habits were not generally affected. The authors conclude that sidedness is not unitary in character and that the central balances related to different aspects of laterality are neurologically not a unitary system. Akelaitis, Risteen, Herren & Van Wagenen (16) studied eighteen epileptics following partial or complete section of the corpus callosum and found no evidence of dyspraxia or apraxia save in cases where other damage was demonstrated in the hemispheres themselves.

Peterson & Chaplin (17) have concluded that the pyramidal systems of the cerebral cortex are the prepotent mechanisms in the control of handedness in the rat. Forty-six subjects were used in their investigation; the effect of lesions in the cerebral cortex, the cerebellum, and the basal ganglia was investigated.

Perception.—Human patients under prolonged periods of refrigeration may remain alert and oriented with rectal temperatures at 93°F. as demonstrated by Fay & Smith (18). Amnesia typically develops for events which occur when the body temperature is maintained at 92°F. or lower. Vocal responses usually failed when the body temperature was reduced below 80°F.

According to Lashley (19), when simple geometric patterns were exposed so that a part fell within a scotomatous area associated with a migraine attack, the patterns were perceived as complete figures in accordance with Fuch's "principle of completion." Such observations rule out the possibility of explaining Fuch's principle in terms of learning.

Harrower (20) has demonstrated that patients with cortical injury show a marked loss in their facility to reverse variations of the Rubin face-vase reversible figure. Since the original discriminations were made effectively, there is no reason to think that the loss results from impaired visual acuity. It is assumed that the deficit results from extra-striate lesions. Lashley (21), on the other hand, reports that in the case of rats, extra-striate lesions produce no loss in conditional responses and concludes that this demonstrates an autonomy of the visual cortex in regard to the functions tested in the particular animals.

Inattention to the left half of external space and consequent selection of right instead of left turnings following parietal lobe lesions in the right hemisphere is described by Brain (22). This disorder was not caused by defect in visual acuity.

A critical interpretation of the syndrome of finger agnosia, characterized by agraphia, acalculia, and disorientation for right and left of the patient's body is presented by Gerstmann (23), who emphasizes that the lesion is localized in the transitional area of the lower parietal and middle occipital convolution of the dominant hemisphere. The case of a patient suffering from anosognosia and autotopagnosia is reported by Olsen & Ruby (24). Of particular interest in this case is the fact that there was no evidence of any

thalamic lesions, characteristically described as occurring in anosognosia.

Riddoch (25) reports that phantom limbs are at first correctly localized in terms of their former position but that later the phantom extremity seems to approach the stump and finally to fade away. Phantom limbs may occur with lesions at different anatomical levels of the nervous system.

Personality.—Pennington & Berkovitz (26) suggest the possibility that impaired performance in the rat following cortical injury may be related to lowered motivation, and that this loss can be partially compensated by increasing the drive intensity.

Removal of either the anterior or the posterior half of the cerebral cortex was not attended by increased "savageness" in rats according to the study by Beach (6). Bilateral destruction of the temporal lobes in monkeys was found by Klüver & Bucy (27) to produce profound emotional changes characterized by lack of aggressiveness toward objects and absence of anger and fear reactions.

Citing three illustrative cases, Harrower-Erickson (28) points out that organic lesions of various types may produce differential symptoms of personality change in children. The same author (29) has carried out extensive investigations testing patients with cerebral tumors by means of the Rorschach test. The so-called "organic" cases may be differentiated from nonorganic cases showing clinically similar symptoms. The outstanding characteristics of the records of patients with tumor are their uniformity and constriction. The total number of responses is small and the relative number of F− (bad form) responses is high. Lack of response on the affective side of personality is indicated by small output of C, CF, and FC (color and combined color-form responses) and c and K (responses to shading). These losses continue postoperatively even though the patient may have a high Binet score and show evidence of an "abstract attitude." Patients suffering from focal epilepsy show greater variability in Rorschach pattern (30). This variability in pattern makes possible differential prognosis which may be used with considerable success to predict the likelihood of postoperative improvement.

Reduction of anxiety, obsessive states, and depressions following mutilating operations involving the frontal lobes have been

reported by Worchel & Lyerly (31) and by Freeman & Watts (32). The detailed accounts by the latter workers show their patients following operation to be the victims of shallow childlike emotions. These emotions are often violent and uncontrolled and are characterized by "a certain affective incontinence." However, the patient is relieved from the protracted, planned, foresightful emotional states which beset the adult life of man. Both the preoperative and postoperative Rorschach psychograms show a poverty of well-integrated and well-adjusted emotional responses and unusual indication of explosive, uncontrolled emotionality. The composite record gives no indication of postoperative improvement.

Visual functions.—Bridgman & Smith (33) report that partial destruction of the striate cortex in the cat produces a fivefold elevation of the brightness threshold and that ablation of the striate area raises the brightness threshold five hundred times. These data show that partial corticalization of the described function has taken place in the cat. A detailed, analytical, and prolonged investigation of the effect of removal of the occipital lobes upon visual functions in the monkey is described by Klüver (34). Absolute and differential brightness thresholds obtained by a number of different experimental conditions are given. The bilateral occipital monkey may make differential responses on the basis of the position of the brightness pattern. Differential responses cannot be made on the basis of brightness *per se*, but can be made only to differences in the density of luminous flux entering the eye. Topographical aspects of the stimulus configuration, with luminous area and brightness constant, may become effective in determining the reactions of the animal. The scotopic thresholds at different wave lengths are similar to those obtained for man. No evidence for color vision was obtained. Additional data on the functional significance of the geniculostriate system in the rhesus monkey are presented in a later paper (35). Elimination of the geniculostriate system leads to "an elimination of visual space with its dimensions" . . . and " . . . wave length itself is no longer an effective determinate" in providing cues for differential responses. No evidence is found that "engrams" laid down by specific training on light stimuli "deteriorate more readily with the passage of time in animals lacking the striate cortex than in intact animals."

Lashley (36) has presented a theoretical account of the nature

of cerebral organization and action in relation to visual functions. The suggestion is made that in regard to certain of the simpler phenomena of visual perception the stimulus is integrated into a relatively simple pattern of excitation in which the dominant feature may be a directional association of elements, a sort of polarization.

Smith (37, 38) has studied in the guinea pig the effect of various cortical ablations, including bilateral removal of the occipital areas, hemidecortication, and complete decortication, upon the nystagmic responses to moving striated visual fields. Cortical operations produce no change in the upper threshold velocities of movement vision, but result in some increase in frequency and magnitude of nystagmic head responses and in an increase in extinction time of optokinetic nystagmus. Bilateral destruction of the striate areas in the cat produces few significant changes in optic nystagmus, tested by means of an alternating black and white striped revolving drum, according to Smith, Kappauf & Bojar (39). Similar data are reported concerning nystagmic movements to apparent as opposed to real movement, measured by means of the revolving drum technique. Stroboscopic movement of striated visual patterns elicited consistent head nystagmus in decorticate guinea pigs, even though frequency of response was definitely increased and amplitude somewhat decreased (40). Both normal cats and cats tested following removal of the striate areas gave appropriate optic nystagmic responses to changes of direction in apparent movement. Responses in the normal animals were somewhat more consistent (41). The various experiments are interpreted as indicating that the cerebral cortex in subprimate animals is not essential to pattern vision *per se*, but only to pattern vision to single moving objects or stripes. Klüver (35) has contended, however, that the existence of optokinetic responses is not an adequate criterion for the existence of pattern vision.

Auditory functions.—French (42) tested rats for the effect of lesions within and without the primary auditory area on the discrimination of simple auditory rhythms. Lesions outside the auditory area did not appear to affect the difference limens, a finding in keeping with that reported by Lashley (24) concerning a visual perceptual problem. Extensive lesions of 70 per cent or more of the cortex lying outside the auditory areas did affect retention of the problem. Extensive lesions, over 85 per cent, within the auditory

area did seriously affect the difference limen. Limited evidence for a mass action relationship was obtained. W. A. Hunter (43), on the other hand, found no evidence that lesions in a "critical auditory area" in the temporal region of the rat brain interfered with the learning of a simple auditory conditioned response. No evidence was found that lesions outside the critical auditory area produced any amnesia for the problem. Differences in results in the two investigations may be explained on the basis of the greater difficulty of French's problem. Furthermore, the lesions reported by Hunter never involved more than 52 per cent of (Pennington's) "critical auditory area" nor more than 27 per cent of the cerebral cortex.

A case of pure word deafness is described by Hemphill & Stengel (44). The patient retained serviceable hearing as indicated by an audiogram and possessed fluent articulated speech without paraphasias. Visual comprehension of language symbols, the ability to understand complex written commands, to name objects, and to write and spell correctly was not lost. The pure word deafness was not complete since the patient could understand some things "close to his heart" especially when they "concerned his normal sphere of life." He understood his wife and friends more easily than strangers. Failure to respond to auditory danger stimuli is interpreted in terms of an associated pain asymbolia, but the possibility of auditory imperception is hardly excluded.

Olfactory functions.—Allen (45, 46) has tested the effect of various cerebral extirpations on positive, negative, and differentiated olfactory conditioned responses. Large extirpations of the parietal, occipital, and temporal (exclusive of the hippocampi and pyriform areas) lobes had little or no effect on any tests. Bilateral frontal lobectomy abolished conditioned olfactory differentiation between clove and asafetida in all dogs. Bilateral extirpation of the pyriform-amygdaloid areas likewise abolished conditioned differentiation without affecting positive conditioned responses to clove or discriminations between food and no-food made on the basis of olfactory cues. Inclusion of the hippocampi with the pyriform-amygdaloid ablations resulted in no additional deficit.

Learning and intelligence.—Carlson (47) reports that thyroidectomy increases error and time scores and reduces retention of maze learning in rats. Daily injection for twenty-four days of metrazol, sufficient to induce death in some animals, produced no

differences in the subsequent learning of a multiple T-maze by rats, according to Bunch & Mueller (48). Biel & Wickens (49) found that rats suffering from a vitamin B_1 deficiency gave significantly poorer results on conditioned eyelid reponses than did litter mate controls.

The effect of curare and erythroidine paralysis upon the learning process is reported by Girden (50). Conditioned rage responses were obtained when the paralysis was incomplete and this learning was dissociated from the behavior of the animal following recovery from the drug.

Harlow & Bromer (51) have described the formation of either conditioned or pseudoconditioned responses in the monkey during an interval in which the motor, premotor, and somesthetic cortex was inactivated by freezing with ethyl chloride. These data are interpreted as indicating that neither a postural substrate nor proprioceptive traces are essential for the formation of simple acquired responses.

Comparing a group of normal children with a group of spastics matched for sex, chronological age, and mental age, Cotton (52) noted on the part of spastics a greater tendency toward concreteness and stereotypy and less ability to shift to more abstract forms of response.

No differential effect between distributed and massed practice was found by Ericksen (53) in operated rats which failed to learn a maze. Successful subjects, both normal and operated, made fewer errors with longer intertrial intervals, but made a greater percentage of repetitive errors. The hypothesis is advanced that massed practice may produce greater variability of behavior.

Maier (54) reports that the repeated testing of rats in discrimination situations decreased the number of equivalent stimuli, whereas cerebral injury increases the number of equivalent stimuli. The assumption is made that cerebral injury decreases reaction to detail so that general and relative features become more important.

Strauss & Werner (55), using a variation of the Halstead sorting test and a picture-multiple object combination test, conclude that the reason why children with brain injury select more objects for combination in all tests is that they have a tendency to abstract many details. Disinhibition is given as another possible reason. These authors stress the fact that brain-injured defectives see pictures as representing concrete elements in a situation, rather than

as abstract pictorial things. Werner & Bowers (56) tested auditory-motor organization in brain-injured and congenitally mentally deficient children. The responses of the brain injured group were the less adequate.

The effect of lobectomy upon learned behavior continues to attract considerable attention. A detailed description of the effects of partial and total destruction of the temporal lobes of monkeys has been presented by Klüver & Bucy (27). Bilateral temporal lobectomy is characterized by "psychic blindness," oral tendencies, "hypermetamorphosis," or forced reaction to environmental stimuli, loss of fear and anger responses, and hypersexuality. Serious sensory or motor changes did not occur and the monkeys were able to learn simple visual discriminations after considerable training. No other operative procedures produced the syndrome save possibly the interruption of the occipital-temporal connections.

The effect of extirpation of the right temporal lobe in man is reported by Hebb (57). The tested I.Q. was 113 and comparable scores were made on other language tests. Responses on nonlanguage tests were uniformly poor and "the defects with performance tests are in some ways like a loss of 'abstract behavior'." In spite of this loss the patient displayed an alert responsive manner commonly associated with "inactive" lesions.

Hunter & Hall (58) trained rats to effect double alternation on an appropriately arranged ten unit block maze. The somewhat limited success enjoyed by three subjects was abolished by bilateral prefrontal lobectomy.

Monkeys may solve delayed reaction problems, according to Finan (59), if pre-delay reinforcement is given, i.e., if the monkey receives a single small food reward placed over the correct container before the beginning of the delay interval. After fairly prolonged training one of the two subjects responded successfully without the aid of pre-delay reinforcement. Finan interprets the contribution of the intact frontal lobes as being that of a derived reinforcing agency.

Using an indirect method of testing for delayed reactions in the monkey, Malmo (60) claims to have obtained delayed reactions following bilateral removal of the frontal lobes. The "indirect method" of delayed reaction training was used. Illumination of the cage during the delay interval produced a transient interfer-

ence postoperatively in the one subject tested for a considerable period of time.

Hebb & Penfield (61) report a case of extensive bilateral removal from both frontal lobes of a man following trauma with subsequent epileptic attacks. The operation resulted in marked intellectual and affective improvement. Postoperative Binet intelligence was normal for his social group, the "ingenuity" problems of the Stanford-Binet test were solved, performance test scores were normal, and no loss is reported for limited tests of "abstract behavior." The suggestion is made that mental changes described by Goldstein as being typical of prefrontal cases "are the product of cerebral dysfunction" and a similar explanation is given for differences between their case and that reported by Brickner. By way of interpretation the suggestion is made that,

> It may be that only in such features as learning in social situations, in adaptation to drastic environmental change or in initiative and the ability to plan and organize one's affairs may be found the impairment that we believe must exist after large lesions of the frontal lobes, but of which we have found no evidence in the present case.

More typical symptoms have been reported by Nichols & Hunt (62) in a case of partial bilateral frontal lobectomy in man. In spite of the fact that an I.Q. of 120 was found and that the patient did well on Goldstein's color categorization test and adaptation of the Kohs' block test, various indications of defect appeared. Analysis of the Vigotsky test showed failure in "consideration of the total system;" failure spontaneously to devise little tricks of technique and to profit from his errors by inventing new methods of attack was observed. Such indicators of lack of spontaneity, rigidity, and failure to integrate a number of factors simultaneously operating are not in keeping with tested intelligence or with the subject's history before the onset of the disease. The report cites evidence for postoperative recurrence of the tumor but discounts the possibility that its extent was great enough to produce pressure symptoms.

Worchel & Lyerly (31) have noted intellectual deterioration in ten patients following prefrontal lobotomy. Inability of patients spontaneously to name a large number of words in a three-minute period is striking.

Freeman & Watts (32) have reported that general intelligence

(I.Q.) was not found to be appreciably decreased following frontal lobotomy. They concede slight evidence of postoperative intellectual loss on the Rorschach test. Adequate determination of the effects of the operation on intellectual processes is obscured by the poor preoperative scores. Certainly the fact stands out that the composite postoperative score in no way resembles the Rorschach picture of normal people. There is every sign of extreme intellectual poverty. In general, Freeman & Watts appear to recognize that intellectual deterioration has occurred. Reviewing the careful detailed studies of earlier and competent investigators they conclude that previous theory "lacks precision and lacks adaptation to the individual case." For purposes of clarity they suggest the topological hypothesis that,

> The cerebral cortex may be considered as being divided by the Rolandic fissure into two portions of essentially different function. . . . Just as the post-Rolandic cortex is concerned with the past, the pre-Rolandic cortex is concerned with the future.

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BIOLOGICAL ASSAY

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Biological assays may be defined as determinations of potency or toxicity based upon the reaction of living matter, including biological reactions not involving intact cells, such as serological tests *in vitro*. Qualitative assays for the indentification of a substance through a characteristic reaction precede quantitative tests but will not be considered here. The main advances have been in the development of quantitative bioassays, whether used as research tools or for routine standardization, through the collaboration of statisticians and experimental biologists. Since the recent literature includes many more papers than can be mentioned here, it seems more useful to consider the general principles of biological assay and refer only to the papers which illustrate them conveniently. In many cases our conclusions are based upon an independent analysis of the published data and do not necessarily agree with those of the original author. Few references will antedate the texts by J. H. Burn (49) and by K. H. Coward (64) to which the reader is referred. Attention should be called especially to the monograph by Ipsen (275) on the theory of biological standardization based upon experiments with bacterial toxins.

Agricultural poisons, viruses, antitoxins, vitamins, hormones, and drugs of all types as well as physical agents, such as x-rays, have been utilized in developing the theory of biological assay although few of these fields can be more than mentioned. We will use the term "drug" as representative of them all in the following discussion. While chemical or physical methods are displacing some biological assays, for many questions the reaction of the living organism remains the final arbiter.

The characteristic reaction to a given drug is usually preferred to a more general response for measuring its potency. This has led to the introduction of the various animal units of dosage, such as

the "cat unit" for digitalis, the "pigeon unit" for prolactin, and many others. These biological standards have proved so variable that wherever possible they have been replaced by standard preparations and the unit defined as the activity represented by a stated amount of the reference standard. The definition in "units" of response sometimes has implied a direct proportionality between the response and the amount of drug in the sample. This is seldom true, as has been shown, for example, by Miller (200) in the assay of parathyroid extract from the serum calcium of dogs.

Even though the true relation between dosage and response is determined in the form of a dosage-response curve for the reference standard, this cannot be used directly for converting any given response to units of standard drug. Most biological indicators have so unstable an average susceptibility that parallel tests on both the reference standard and the unknown sample are essential for reliable estimates of potency. Of the cases which have been examined critically, the only exception is the mortality of the eggs of *Drosophila melanogaster* to x-rays, which has served as a biological indicator of dosage (34). Eggs from several stocks were tested with the same Coolidge tubes over seven years with an average of 275 eggs in a lot. The individual observations agreed with the standard curve within the sampling error; this fact indicates a stable susceptibility which is unique in the field of biological assay. Although the variability of most biological units has discredited them for the control of drug uniformity, they are still used widely in research on new preparations and have contributed to the confusion characterizing assays of endocrine secretions especially.

Relative Precision in Assay Procedure

Stable reference standards have been adopted for the more important drugs which must be controlled biologically. Each is distributed for assay purposes from a central agency, such as the Board of Trustees of the U. S. Pharmacopoeia and the National Institute of Health. Except for the official preparations, there has been less progress in the adoption of suitable drugs in each class as research standards for controlling the variability of biological indicators.

Methods for using standard preparations.—After its approximate potency has been determined by chemical or physical methods or by preliminary biological assay, a drug is tested together with the

standard under as nearly identical conditions as possible. The objective is to determine how much of the sample or unknown will produce the same biological effect as a known dose of the standard. Three procedures have been in general use for establishing relative dosage.

The first are assays primarily of minimal potency as typified by those in the U. S. Pharmacopoeia for vitamins A, B_1, and D. These stipulate that the reaction to the unknown must equal or exceed that to the standard at a given dose. A recent collaborative study on the vitamin A content of the present U.S.P. Standard (255) illustrates the variability that has existed in minimal assays of this type. This assay is based on the gain in weight of young rats. If two groups were tested with the same dose of a single oil, their mean responses would seldom coincide so that in half of the assays the mean gain on one would be expected to fall short of the other. If the latter were the working standard, the assay would not meet U.S.P. requirements. Hence, minimal potency assays have led to the addition of a variable excess for the needs of check assays. Despite their use of a standard, such tests of potency are incomplete in that they provide only a lower limit. A definition of "maximum potency" has been added in U.S.P. XII by a second single-dose assay at a higher assumed level, but its use still does not provide a quantitative estimate of the most probable potency of a given preparation.

A second class provides for repeated adjustment of the dosage of the sample until it produces a submaximal response equal to that of the standard or vice versa. The definition of "equal response" is one source of difficulty in assays of this character. British usage for the oxytocic principle of posterior pituitary extract has been to approach the activity of one preparation by adjustment of the concentration of the other from both above and below, narrowing the range enclosing an equal response to a dosage ratio of less than 1.5 and averaging the potencies corresponding to the smallest range. From the variation between two or more independent tests on the same sample of extract, Gaddum (116) has estimated the standard error of an assay with one uterus at 7.7 per cent.

The U.S.P. XII has adopted the method proposed by Smith *et al.* (241) for posterior pituitary, requiring that a series of four similar contractions obtained alternately by one dilution of the standard and one of the unknown be followed by a 25 per cent

larger concentration of the standard. The height of each contraction is then measured. The first four contractions are considered equivalent if the difference between the highest and the lowest of the four is less than half the difference between the lowest and the height of the fifth contraction, resulting from the larger dose of the standard. The frequent failure of a uterine strip to produce an acceptable assay of the oxytocic principle has led Morrell *et al.* (212) to redesign the assay as an all-or-none procedure. Although assay by successive approximation has been used for diverse substances, such as epinephrine (U.S.P. XII), adrenal cortical hormone (254), and laxatives (184), it is feasible for only a small number of drugs and has other limitations. It does not test the qualitative equivalence of the standard and unknown, provide an inherent check on its own error, or use objectively the many preliminary "approach" tests which commonly precede the four or five reactions constituting an acceptable result. It is especially inefficient and unsatisfactory as a method for determining the so-called "threshold dose."

The third class of assay with which we are primarily concerned is based upon an empirical determination of the relation between the response and two or more different doses of drug. In some instances a standard dosage-response curve has been established at the outset and used thereafter for estimating potency from the response to a single dose of standard and to a supposedly equivalent dose of the sample. This assumed that the slope of the dosage-response curve is constant even though its position may fluctuate. The slope of a curve has usually proved considerably more stable than its position (91, 30) but yet sufficiently variable to warrant testing it as an integral part of most assays. Hence the recent trend has been toward a multiple-dose technique following a pattern based initially upon the nature of the response and its relation to the dose of drug.

Specificity of response.—Preparations of constant therapeutic potency cannot be insured by the design of the assay alone. Only when the composition of the drug is qualitatively constant is a biological assay equivalent to that obtained by weighing the isolated principles. Thus the specificity of the biological indicator and the presence of interfering substances assume great importance. Factors modifying the sensitivity of a response are controlled supposedly by the use of a standard, but this does not adjust the interfering factors in the preparation under test. Hence, it is evi-

dent that the statistical evaluation of assay results, such as those presented in the tables of this paper, do not assure their qualitative validity.

In the bioassay of vitamin D in milk by the rat bone-healing technique, the addition of factors such as phosphorus or skim milk greatly increased the response (209), from which it has been concluded that in food assays for vitamin D the result is an over-all response of the experimental animals to a nutritional complex. Similarly Coward & Kassner (65) found that vitamin D with additional phosphate produced a greater healing than that expected from the separate treatments, and concluded that in assaying food substances containing phosphorus, the ether-soluble portion should be extracted for the determination of its vitamin D content. The salt intake also affects the response to vitamin D (129).

The discovery of ergonovine and its importance has shown the inadequacy of the U.S.P. assay for ergot since the proportion of ergonovine varies considerably between extracts and its action differs from that of other constituents. As noted by R. G. Smith (244), the uterine-stimulating action of ergonovine of therapeutic value exceeds that of the ergotoxine group, but this difference is not reflected adequately by the cock's comb reaction. Similarly, ergonovine has almost no effect in the Broom-Clark method, which is based upon the inhibition of the effect of epinephrine upon the uterus of the rabbit.

Specificity in response has an important bearing in the assay of posterior pituitary extracts which contain both pressor and oxytocic principles. Coon (62) has described an assay of the oxytocic principle utilizing the blood pressure of the chicken. With this method comparative assays have given pressor-oxytocic ratios of pituitary solution two and a half times higher than the value obtained by the U.S.P. guinea pig uterine method, which led him to believe that in preponderant amounts the pressor component inhibits the uterine action of the oxytocic constituent. Later R. B. Smith (243) found that the pressor principle does not inhibit the action of the oxytocic principle but augments the depressor response. Further experiments traced this to the higher concentration of magnesium in chicken blood.

Two other cases may be cited. When the present crystalline International Standard of insulin was assayed collaboratively against the older, amorphous standard, two methods were used,

the incidence of convulsions in mice and the hypoglycemic response in the rabbit. Within each method the agreement was good, but the first indicated a potency of 25.2 ± 0.33 units per mg. and the second a significantly lower value of 21.5 ± 0.16 units (175). Although toxicity has often been used as an index of therapeutic potency, Morrell & Allmark (211) reported that in commercial neoarsphenamines, trypanocidal potency varied inversely with toxicity to the rat.

When relative potency differs with the test object, as in the above examples—and there are many others in the literature—it becomes a matter of prime importance to know which of several potencies represents the therapeutic value of the drug. The relative potency of various estrogenic substances varies with the method of assay. Although their therapeutic potencies have yet to be established quantitatively, it is clear that estimates based on animal methods are not always applicable to man. For example, Shorr *et al.* (238) have contrasted the greater potency in man of ethinyl estradiol than of stilbestrol with the results of animal assays (90) in which stilbestrol was three times the more active. Freed *et al.* (111) have reported that in patients in the menopause with moderate or severe symptoms, diethylstilbestrol dipalmitate is much more potent than diethylstilbestrol although in the rat the latter has fifty times the greater effectiveness.

One of the most striking discrepancies is seen in the relative effectiveness of the digitalis glycosides in different test situations. When assayed by the intravenous cat method, lanatoside C is somewhat more active than digitoxin (126, 168). Its therapeutic potency by oral administration in man, however, is only about a tenth that of digitoxin. In man the material must first be absorbed from the gastrointestinal tract, whereas the cat assay method involves an intravenous fatal dose. Differences in absorption or destruction in the gastrointestinal tract cannot account for all of the discrepancy since lanatoside has proved less potent than digitoxin in isolated preparations of the chick embryo heart (78) and of cardiac muscle (54). Such observations suggest that different animals metabolize the glycosides of the digitalis group in a qualitatively different manner. For these reasons the necessity for therapeutic standardization by direct test in man is receiving increased recognition (85, 124, 125, 238).

Role of collaborative assays.—Assuming that the technique for

individual assays has been studied adequately in one or a few laboratories, collaborative tests may have several objectives. If one "unknown" sample is a known dilution of the standard, not disclosed until the end of the assay to avoid bias, the potency determined biologically may be compared with the true value to test the accuracy of the assay. From the combined result of several to many repetitions using a standardized procedure, the technique can be checked for the consistency between replicates and for bias. With two or more replicates in each of several laboratories, the adequacy of the definition of the method can be tested.

Few collaborative assays have met these requirements. Accuracy has seldom been measured independently of precision through failure to include two different concentrations of the same preparation, or collaborators have differed in the number of doses used with each preparation and the number of animals on each dose (13). In many cases details of technique have not been standardized sufficiently before starting the experiment so that the source of discrepancies could not be identified. Thus the relative potencies of several gonadotrophic hormones, obtained by one or more methods in each of 32 collaborating laboratories, have been compared statistically by Emmens (92). His analysis indicates the relative precision of four or more techniques as actually used in this study but not necessarily their rating if each technique had been standardized.

The League of Nations study on the vitamin B_1 assay (176) approached the ideal more closely, and the recent collaborative studies sponsored by the Revision Committee of the U.S.P. on both the frog and cat assays for digitalis (201) have been equally productive of quantitative and comparable results. These experiments were designed so that the relative potencies and their precision could be computed from the results of each collaborator. A comparison of the mean results from different collaborators then revealed any discrepancies which could not be accounted for by the experimental errors occurring within each laboratory. Finally, the combined results could be checked for accuracy by comparison with known relative potencies where the samples were different concentrations of the same preparation. Such designs are vital in the development of bioassay procedure, especially where used for the control of drug potency.

The error of individual assays.—Before an adequate collabora-

tive experiment can be designed, the individual assay must be quantitative and have a determinate error. Gaddum (115), Bliss (25, 33), Irwin (155), and their collaborators have developed the general statistical methods appropriate for individual assays. These have been based directly or indirectly upon the curve relating dosage and response, which largely determines the inherent precision of any given technique. The steeper this curve, the better differences in dosage or potency can be discriminated.

The recent trend has been to convert the observed response, where necessary, to a unit which can be plotted as a straight line against the logarithm of the dose. The transformation used in rectifying all-or-none or quantal assays has had a theoretical basis such that the reciprocal of the slope is the standard deviation of a single observation for the threshold log-dose. Hence the inherent precision of a given all-or-none procedure is best expressed in terms of this standard deviation or $1/b=\lambda$, the symbol introduced by Gaddum (115) to refer to the standard deviation of the log-dose inferred from the straight line relating the response to the log dose. For dosage-response curves without the theoretical base of the quantal assays, the standard deviation measuring the variation in response about the curve (s) is converted to units of log-dose by dividing by the slope (b). Hence for all other assays the ratio $s/b=\lambda$ is the corresponding estimate of inherent precision in terms of the standard deviation for one observation. The term λ will be used here for summarizing the numerical values.

These estimates of the standard deviation assume that the data are consistent with a straight dosage-response relation within the range of the assay. For quantal assays, the agreement between observations and hypothesis could be tested by χ^2 (28). For other types, two tests are involved. The first determines whether the observed relation between dosage and response is curved sufficiently to discredit the use of a straight line, for which a simple application of the analysis of variance may be used (33). The other tests whether the standard deviation in response at the different dosage levels of an assay is sufficiently uniform to warrant computing a pooled estimate applicable to all dosages. For doubtful cases, Bartlett's (16) χ^2 test for the homogeneity of a series of variances (variance being equal to the square of the standard deviation) is of value. The computation of an assay when the standard deviation as well as the mean response is a function of dose will be discussed later.

Although dosage is estimated from response by means of the dosage-response curve, it is essential to compute the curve relating response, the dependent variate, to dose, the experimentally-determined independent variate. Eisenhart (88) has discussed the necessity for this approach rather than the reverse technique of calculating the dose as a function of the response, which has been followed incorrectly in some recent work (109, 160).

One of the most important qualifications for an assay procedure is that the dosage-response curves for standard and unknown must be parallel when dosage is expressed in logarithmic units. Given slopes agreeing within the experimental error, the two curves can then be moved horizontally until one coincides with the other. A single dilution factor, determined from the difference on the logarithmic axis between the two curves, is then sufficient to equilibrate the two preparations in terms of the response used in the assay. If the slopes are not parallel, either the test with the standard differed in some essential experimental detail from that with the unknown so that they are not comparable, or the two preparations are not qualitatively alike in their physiological action. Thus Shrimpton (239) has determined that in the bone ash assay for vitamin D in rats, the slope of the dosage-response curve for butter exceeded that for the standard vitamin D oil significantly. Marks (194) has observed a smaller slope for crude preparations of insulin than for the crystalline standard in the mouse assay. With such qualitative differences, the estimated relative potency varies with the level of response and it may be necessary to separate the active principle from the components which modify the reaction. If the assay technique is not changed, the level of response used in computing relative potency should be given.

The necessity for parallel dosage-response curves holds only when dosage is expressed in logarithms. Occasionally the response has proved a linear function of the dose rather than of the log-dose. In such instances the curves for preparations of unequal potency should differ in slope but converge to zero dose. The ratio of the slopes is then a measure of relative potency. Such assays are exceptional and their statistical treatment has yet to be described. O'Brien & Morgareidge (214) plotted the degree of healing in rachitic rats directly against the dose of vitamin D in the presence of different amounts of phosphorus and obtained a series of straight lines differing in slope and converging to zero dose. The slopes of

these dosage-response curves, termed the "enhancement factor," could be plotted linearly against the daily dose of phosphorus. Using the rat bradycardia technique, Harris (176) determined the relative activities of crystalline and of absorbate standards of vitamin B_1 from the relative slopes of the two straight lines for length of cure in days, plotted against the dose of drug. A similar linear relation has been observed between the log-growth of lettuce root-tips and length of exposure in the assay of depth doses of x-rays as reported by Henshaw & Francis (145). However, in subsequent references to the dosage-response curve, we will assume that the dosage is expressed in logarithms.

In studying the dependence of quantitative biological assays upon the relation between dosage and response, the aims have been to widen the range of response available for assay purposes, to make more effective use of biological material, and to compute objectively the potency of a sample and the precision of the assay. The design of the assay and its computation have depended largely upon the characteristics of the response used as the indicator of potency or toxicity. These responses have been classified in three main groups, which may be considered in order.

Assays Based Upon An All-or-None Response

Many reactions have been used as biological indicators (Table 1) in all-or-none or quantal assays. In each case potency is assumed to be inversely proportional to the smallest amount of drug or "threshold dose" which will produce the characteristic response. As with other biological measurements, the threshold dose varies from one animal to another so that it must be determined for groups rather than for isolated individuals. Two methods are in common use: (*a*) direct estimation of the threshold dose for each individual in the group by titration with the drug, such as with cardiac glycosides in cats, and (*b*) indirect estimation from curves based upon the percentage of positive reactions in separate subgroups tested with different doses of drug.

Direct estimation of the threshold dose.—Except for a recent paper on mercurial diuretics (77) the direct estimation of the threshold dose in each individual has been confined largely to the intravenous infusion of digitalis and other cardiac glycosides in cats (U.S.P. XII), guinea pigs (2), dogs (23), and other animals. Though the measured dose increases at faster rates of injection

TABLE I

DATA ON OBSERVED PRECISION OF VARIOUS ALL-OR-NONE ASSAYS. SUPPLEMENTING TABLE I IN GADDUM'S REPORT (115).

Drug	Animal	Response	Slope, b	$\lambda = 1/b$	Reference
Vitamin B_1	Depléted rat	Cure of polyneuritis	3.55±0.88	.282	Supplee *et al.* (247)
Vitamin B_1	Depleted pigeon	Cure of polyneuritis	1.66±0.37	.601	Coward & Morgan (68)
Vitamin E	Depleted rat	Litters per mating	4.99±0.38	.200	Hume (152)
Insulin	Mouse	Convulsion	3.52±0.15	.284	Marks & Pak (195)
Gonadotrophic hormone	Dietary anestrus rat	Cornified cells in vaginal smear	8.72±1.03	.115	Heard (138)
Gonadotrophic hormone	Post-partum rabbit	Ovulation	28.59±6.88	.035	Friedman (112)
Estrone	Ovariectomized mouse	Cornified cells in vaginal smear	5.46±0.52	.183	Emmens (91)
Lactogenic hormone	Guinea pig	Lactation	1.31±0.32	.763	Lyons (188)
Stilbestrol	Immature rat	Introitus opening	6.09±0.67	.164	Lee *et al* .(177)
Posterior pituitary	Guinea pig uterus	Contraction	2.7 to 17.1	.37 to .058	Morrell *et al.* (212)
Vasopressin-oxytocin	Laying hen	Premature oviposition	3.05±0.41	.328	Burrows & Fraps (50)
α-Staphylysin V/37	Rabbit blood cell	Haemolysis	2.75±0.09	.363	Ipsen (275)
Tetanus antitoxin*	Inoculated mouse	Death in 6 days	32.2 to 69.1	.031 to .014	Smith (242)
Papilloma virus	Rabbit	Wart development	1.34±0.17	.744	Bryan & Beard (45)
Neoarsphenamine	Inoculated mouse	Survival	9.17±1.14	.109	Bülbring & Burn (48)
Neoarsphenamine	Inbred inoculated rat	Negative blood smear or 5-week survival	8.70±0.44	.115	Morrell & Allmark (211)
Neoarsphenamine	Inbred rat	Death (acute toxicity)	15.29±0.93	.065	Morrell & Allmark (211)
4 Sulfonamides	Inoculated rat	Survival	5.97±0.77	.168	Litchfield *et al.* (183)
Digitalis	Cat, ether	Death	15.32	.065± .004	Bliss & Hanson (32)
Digitalis	Dog, barbiturate	Death	18.48	.054± .008	Blickensdorfer & McGuigan (23)
Digitalis	Frog	Death, 18 hours	9.49±0.63	.105	Bhatia & Lal (21)
Digitalis	Frog	Death, 18 hours	13.10±0.46	.076	Allmark & Morrell (4)
Digitalis†	Frog	Death, 18 hours	12.07±0.66	.083	Miller (202)
Digitalis‡	Frog	Systolic standstill	7.26±0.62	.138	Miller (202)
Adonis	Pigeon	Emesis	13.44±2.41	.074	Benson & Edwards (19)
Squill§	Rat	Death	3.19±0.87	.313	Ward *et al.* (262)
2 Barbituric acids	Mouse	Hypnosis, Death	13.77±1.29	.073	Foster (105)
Cocaine‖	Mouse	Death	6.06±0.93	.165	Gurd & Sachs (131)
Mercupurin	Cat	Death	9.17	.109± .016	DeGraff & Lehman (77)
Senna	Mouse	Soft stool	7.81±0.80	.128	Geiger (120)
Carbon tetrachloride	Round worm	Death in rat	7.14±0.68	.140	Whitlock & Bliss (266)
Diethylene glycol	Guinea pig, rat, mouse	Death	7.70±0.90	.130	Laug *et al.* (173)
Glycinonitriles	Aphid	Death	2.58±0.08	.388	Moore & Bliss (208)
Red cuprous oxide	Fungal spore	Failure to germinate	4.86±0.29	.206	Wilcoxon & MacCallan (268)
X-rays	Drosophila egg	Death	3.82±0.04	.262	Bliss & Packard (34)

* International standard.
† Composite from four laboratories with steepest average values in U.S.P. Digitalis Study, 1939–40 (202).
‡ Composite from same laboratories as footnote † (202).
§ Based upon deaths during first three days after treatment.
‖ Computed from doses of 0.1 mgm. per gm. or larger.

(86, 259) and varies with the anesthetic (2, 113, 135), comparable results have been obtained with injection periods of one to one and one-half hours and uniform use of a single anesthetic.

The threshold dose for a stable preparation of digitalis has been analyzed for 52 groups, each of four etherized cats (32). When doses were expressed in logarithms, they were distributed symmetrically about the mean, in good agreement with the normal curve of error. Hence the dosage corresponding to the mean log-dose estimated the LD50. The standard deviation within groups, reported as λ in Table 1, proved to be significantly less than that between groups; this fact demonstrates the potential instability of the so-called "cat unit" for digitalis. A later report from the same laboratory compared the toxicity in etherized cats of twelve cardiac glycosides in parallel tests (57). Ten of these had been tested previously on separate groups of cats at different times, and of the ten, four differed significantly from the earlier determinations in the same laboratory, one by 35 per cent; this finding illustrates the importance of standards and assay procedures in research comparisons of new drugs.

Estimation of the threshold dose from percentage response.—The threshold dose for a quantal response is usually obtained indirectly from the curve relating the percentage of positive reactions to the dose of drug over the intermediate range between the observed 0 and 100 per cent. The resulting asymmetrical sigmoid curve, coupled with the variation unavoidable for the small numbers ordinarily used in each lot, delayed the adoption of quantitative methods.

The most important single advancé was the discovery by Gaddum (115) that this represented a cumulative curve of the biological variation in the threshold dose of drug, which agreed satisfactorily with the normal curve of error if doses were first transformed to logarithms. Since all individuals of a group with an equal or smaller threshold would react to a given dose of drug, he equated the observed percentage response to the accumulated proportionate area in tables of the normal curve to obtain the corresponding deviation from the mean in terms of standard deviations. These standard deviations represented hypothetical "doses" in units which Gaddum called the "normal equivalent deviation" or "N.E.D." Plotting the N.E.D. against the log-dose, the sigmoid curve was transformed to a straight line, which could be computed

objectively and easily. Gaddum gave several pharmacological examples fully illustrating the technique, which was applied in detail by Hemmingsen (140) to the assay of insulin from the incidence of convulsions in mice.

Meanwhile, working independently on insect toxicology, Bliss discovered the same relationship and transformation for the dosage-mortality curve (24). To avoid the change in sign from minus to plus at the 50 per cent point, he added 5 to each deviate and called the sum a "probit." Two later papers (25) described the transformation more fully, proposed a method for weighting each observation that differed in detail from Gaddum's, presented tables for using the transformation, and extended the method to comparisons of toxicity. An appendix by Fisher (25) showed how lots in which 0 or 100 per cent kill was observed could be used in computing the curve.

Certain statistical details in Bliss' procedure were challenged by Irwin (155), which led to a reconsideration of the problem by Fisher. This resulted in the present calculation based upon maximum likelihood (28, 102). The procedure is now recognized as a computation by successive approximations which converge so rapidly to a stable value that the first computed estimate is usually adequate. A provisional dosage-effect curve is placed graphically on a diagram where the tabular or empirical probits for values between 0 and 100 per cent have been plotted against the log-dose. From this provisional curve one reads the "expected" probit for each dosage. These are needed in determining the "corrected" probits for all observations, including those where 0 or 100 per cent is observed, and their weight. More extensive tables to facilitate estimates of the corrected probit and its weight have been prepared (82). The technique and an alternate mode of calculation have been discussed more mathematically by Garwood (118).

The development of basic formulae for applying these techniques to all-or-none bioassays has been completed by Miller, Bliss & Braun (203). Given parallel dosage-effect curves for the standard preparation and the sample or unknown, the distance between them is the log-ratio of potencies designated as M by Gaddum (115) and later writers. Its error depends upon agreement of the observations with the computed curves, the number and distribution of the animals used in the assay, and the slope of the combined curve. The reciprocal of the latter, λ, has been reported for several

all-or-none assays by Gaddum (115) and additional values are given in Table I (pg. 489).

With known doses not controlled exactly and one individual per dose, adjacent doses may be averaged for the provisional estimate and then corrected by the maximum likelihood solution either with or without grouping (28). This technique has been applied by Marshall *et al.* (196) to assays of the therapeutic activity of sulfonamides against streptococcus infection in mice. An alternative calculation has been described by Schäfer (228).

While these methods are flexible and exact, various alternatives have been proposed, primarily to shorten the computation. The simplest is to plot percentages against the dose directly on logarithmic probability paper[1] or, after transformation to empirical probits and logarithms, on arithmetic cross-section paper and fit a straight line by inspection. Without further restrictions as to the dosage interval or the size of each group, Litchfield & Fertig (182) have outlined one approximation and deBeer (72) another for estimating the dosage-effect curve and its error with a minimum of computation. Prigge (219, 220) and Schäfer (227) have developed equivalent methods with varying amounts of calculation and applied them to the assay of biological preparations.

Successive doses are increased preferably by a constant proportion, giving equal log-dose intervals. Where the error could be computed from three complete replicates of each dosage-mortality curve in balanced experiments, Moore & Bliss (208) determined the log-LD95 from empirical probits with equal weights and simple linear coefficients. Marks (194) has described a shortened computation for mouse assays of insulin with three unknowns. Given the additional restriction that dosages cover the range from observed values of 0 to 100 per cent, several simplified methods have been described. Gaddum (115) has discussed a number of possibilities, including tests with one animal to a dose. Wilcoxon & McCallan (268) have given a nomogram for simplifying the calculation in tests on fungicides.

For estimating the ED50 (the effective dose for 50 per cent of the population) several authors (83, 223) have proposed that at a given dose the number of positive responses be augmented by the sum of all positive responses at smaller doses in the series and the

[1] Obtainable from Codex Book Company, Inc., Norwood, Mass., cross-section paper No. 3128.

number of negative reactions by all those failing to react at larger doses. The percentage at each dosage level is then based upon the augmented total of positive and negative effects and the ED50 interpolated from the doses for the two percentages on either side of 50 per cent. If an experiment agrees with the above conditions, this leads to an unbiased estimate of the ED50 but the estimate has an error about six times as large as that claimed recently (270). An alternative procedure, based upon a moving average of adjacent percentages, has been provided with an unbiased estimate of error by Irwin & Cheeseman (156, 157) and applied to tests on anti-pneumococcus serum.

Special applications of the quantal technique.—Several special applications of the log-probit transformation or its equivalent should be noted. In laboratory assays of fungicides by Horsfall *et al.* (151) many details of technique were found to alter the dose per spore and in consequence the dosage-mortality curve, but even with these under control the variation from test to test in the toxicity of red cuprous oxide was reduced by one-half through the use of Bordeaux mixture as a reference standard. Later experiments from the same laboratory (81) assayed fungicides under both controlled and field conditions with especial reference to the slope of the dosage-mortality curve. In several tests separate leaves or plants were scored in five classes from 0 to 4, the scores totaled for each plot, and the sum expressed as a percentage of the greatest possible disease-rating.

Other assays have been based similarly upon percentages not computed directly from the numbers of positive and negative animals. Morrell *et al.* (212) cut the uterus of a guinea pig into eight equal parts and determined the percentage of the eight pieces contracting in different concentrations of posterior pituitary extract. The proportion of successful assays per guinea pig increased markedly over that with other methods although the error they computed from a single assay may be questioned.

Ipsen (275) has identified the degree of hemolysis in the assay of bacterial hemolysins with the variation in susceptibility among blood cells and converted his colorimetric determinations to probits. Changes in the age distribution of the corpuscles through controlled bleedings altered the shape of the log(dose)-probit curve as would be expected if the susceptibility of an erythrocyte to hemolysis varied with its age. By measuring hemolysis at different

times as well as at different doses, Ipsen has developed from the mean resistance (ED50) and the standard deviation ($\lambda = 1/b$) the quantitative relations in the three-dimensional system of dose of lysin, reaction time, and probit response for the assay of toxins.

Bryan & Beard (45) determined the percentage of warts formed after inoculating purified papilloma virus protein in a two-fold dilution series on square inch sites on the back of rabbits. Each rabbit was tested with eleven concentrations on each side and the ED50 determined from the percentage of lesions which developed in thirty-four days.

In a potency test for organic arsenic and antimony compounds, Bülbring & Burn (48) used the survival of mice infected with trypanosomes as the indicator. Ten mice were treated at each of four or five concentrations, and the number surviving recorded for six to nine successive days. To avoid the irregularity in the percentages surviving on any one day, the survivors may be summed over a four-day period, beginning on the first day deaths occur, and transformed to the percentage of possible survivor-days. For neoarsphenamine the dosage-mortality curves had a slope agreeing with that reported by Morrell *et al.* (213) for assays from either a five-hour negative blood smear or a survival period of five weeks in rats infected with trypanosomes (Table I, pg. 489).

Bioassay techniques have been used not only for comparing an unknown preparation with a standard but also for determining the spread between the narcotic and the toxic dose of certain drugs (248). Foster (105) has termed the interval between the dose effective in 99 per cent of a group of rats and that killing 1 per cent of the same or an equivalent group the "standard safety margin." In most cases these levels of dosage would require extrapolation beyond the mortalities observed experimentally (exclusive of 0 and 100 per cent) with a relatively large error of determination, so that limits of five and ninety-five per cent would be more reliable (266).

An additional complication is the change in slope which sometimes has been observed, usually at responses below twenty to thirty per cent (24, 73). In some cases this discontinuity could be attributed to heterogeneity in the stock of test animals (115); to the relation between the concentration of poison and the amount adsorbed within the animal (25); or to the joint but independent action of two active components in the drug or to two relatively independent reaction systems in the animal (29). In the middle dosage range of interest in most drug assays or in the upper range

which concerns the entomologist and phytopathologist, these changes in slope have been of little importance. Certain notable exceptions, however, have been reported in laboratory tests of fungicides (81, 189), some of which have been explained on a physicochemical basis.

Assays Based upon Reaction Time

The indirect measurement of the threshold dose of a drug and of its distribution in a population is less efficient than if the threshold for each individual could be determined directly. In some, but not all, cases (68) individuals receiving more than the threshold have been observed to react with a speed which varies with the dose. Given the record of its reaction time, each individual supplies more information than if classified only as positive rather than negative. Statistically, assays based upon the reaction time of an all-or-none response resemble the graded responses in our third category.

Most reaction-time assays have avoided doses to which only part of the individuals would react. For single doses, Bliss (27) studied the variation in reaction time and reported cases where the logarithm of the time followed the normal distribution. In experiments on narcosis, the upper limit of dosage merges into the range of lethal effect and the duration of narcosis has supplemented the percentages of narcotized and killed individuals at the lower and upper ends, respectively, of the range of narcotizing doses (74, 148, 248).

Quantitative studies have followed one of two hypotheses for correlating these and similar data. The first is that all individuals killed by a partially lethal dose would have required longer to recover than those which survived if the entire group had been given a sublethal dose. On this assumption Broadbent & Bliss (40) computed the mean log-time for the recovery of *Drosophila melanogaster* from hydrocyanic acid fumigation as if the available records at any given dose represented a truncated normal distribution. Since exposure time (t) could be related to hydrocyanic acid concentration (C) for a constant effect by similar equations with either recovery time ($C^{1.7}t = 9.8$) or percentage kill ($C^{1.9}t = 10.2$) as the indicator, they concluded that the two criteria measured the same toxicological process. The second hypothesis is that the time and the occurrence or non-occurrence of a reaction are independent of each other, although both are related to the dose of drug, so that the individuals which fail to react can be disregarded in computing

the mean reduction time. Bryan & Beard (44) have adopted this assumption for studies on the incubation period of positive lesions following inoculation with purified papilloma protein. The mean incubation period in days proved a linear function of the log-concentration and the authors have related it to an alternate criterion of percentage effect in the same experiments. A third possibility, that the correlation between reaction time and the threshold dose within a group is intermediate between the 1 and 0 assumed above, apparently has not been explored.

Starting with the first of these postulates, Ipsen (275) has assayed the potency of toxins from the death time of groups of animals. The relation between the logarithm of the median survival period and the log-dose of toxin over a wide range of doses agreed with the equation

$$(D/d-1)^{\alpha}(T/t-1)=K,$$

where D and T were the observed dose and the survival time, d and t their asymptotes, and α and K constants. Since pure toxins were characterized by stable values of t, α, and K, he transformed the observed death times (T) to a function, $f(T)$, equal to $-\log(D/d)$ or expressed in units of log-dose. The individual values of $f(T)$ at each observed dose were distributed normally with a standard deviation that was reasonably constant in Ipsen's colony of mice. This enabled him to use all death times, including those in groups with incomplete mortality, for assays of toxins that were considerably more precise than if based upon a quantal technique and the same number of animals.

Measurements of reaction-time have been applied in many ways to the estimation of relative potency. Whitlock (265) has compared the survival time for 50 per cent of *Strongylus vulgaris* adults exposed *in vitro* a uniform period of 100 minutes to 0.01 M solutions of four cupric salts. Since each salt was tested at only one concentration, they could be ranked in order of toxicity at that concentration but their relative values were indeterminate. In many biological assays of insecticides, insects have been confined on poisoned food under standard conditions, and their survival time noted. Fleming (103) has shown the importance in tests with Japanese beetles of including a standardized poison in each test and of controlling environmental conditions, but in comparing toxicities at a single dose of each poison (104), he assumed that the

response was proportional to the toxicity. Gersdorff (122, 123) has compared the toxicity of many compounds through the survival time of gold fish under standardized conditions in different concentrations. He has characterized each compound by the minimum product of concentration and mean survival time. However, when the average survival time fell within a range of about one-half to eight hours, the dosage-response curve for log (survival time) against the log (concentration) for several compounds was plotted as a straight line so that the log-ratio of potencies could be computed when parallel curves indicate qualitatively similar action (Table II, pg. 498). Here, as in other research, tests where toxicity is measured from the length of survival in a toxic medium there is the danger of judging the drug from a secondary effect. If drugs differ in their relative potencies when determined from reaction time as compared with the percentage response, the more reliable of the two criteria is that approaching more closely the ultimate use of the drug or poison.

The length of survival is a frequent criterion in the assay of antitoxins and other biologicals. Because of the small numbers involved and the necessity of discontinuing tests while there are still survivors, approximate equations have been described by workers in the State Serum Institute in Copenhagen (245) for determining the median survival time or T50.

Kline *et al.* (164) have determined the dosage-response curve for duration of cure of experimentally produced polyneuritis in the rat plotted against the dose of vitamin B_1. The U.S.P. assay, based largely upon this study, however, is a minimum potency procedure that does not utilize the curve directly. When applied critically, as in the report by Nelson & Kline for the League of Nations collaborative study (176), this technique proved one of the most precise in the entire series. Quantitative biological assays, testing each drug or sample at two or more dosage levels, have been based upon reaction time by several workers. Calloway *et al.* (51) have based an assay technique for one of the pituitary hormones upon the length of time for the melanophores of hypophysectomized frogs to return to full contraction after treatment. When the response fell within a range of 50 to 240 minutes, the time (in minutes) could be plotted linearly against the log-dose. DeGraff *et al.* (78) have compared fifteen chemically related glycosides and genins in terms of the time interval from immersion of the embry-

TABLE II

DATA ON PRECISION OBSERVED IN SELECTED REACTION-TIME ASSAYS

Drug	Animal	Response Time to	Unit of Response	S.D. s	Slope b	λ	Reference
Vitamin B_1	Depleted rat	Recurrence of polyneuritis	Days	1.99	14.0	$.142 \pm .025$	Harris (176)
Vitamin B_1	Depleted rat	Recurrence of polyneuritis	Days	1.22	39.3	$.031 \pm .006$	Nelson & Kline (176)
Vitamin B_1	Depleted rat	Recurrence of polyneuritis	Log-days	.139	1.079	$.129 \pm .020$	Guerrant (176)
Vitamin B_1	Depleted rat	Recurrence of bradycardia	Log-days	.117	.775	$.151 \pm .011$	Leong & Harris (179)
Vitamin K	Blood of depleted chick	Clot formation*	†Log-seconds	.052 to .112	.241 to .711	.158 to .237	Almquist & Klose (5)
Thrombin	Horse plasma	Clot formation	†Log-seconds	.028	.737	$.038 \pm .007$	Jaques (159)
Adrenal cortex extract	Adrenalectomized drake	Death	Days	3.76	17.48	$.215 \pm .036$	Bülbring (46)
11 Cardiac glycosides	Embryonic chick heart	Atrio-ventricular block	†Log-minutes	.199	.814	.245	DeGraff *et al.* (78)
Digitalis	Embryonic chick heart	Atrio-ventricular block	†Log-minutes	.062 to .168	.55 to .151	$.120 \pm .010$	Lehman & Paff (178)
Papilloma virus	Rabbit	Appearance of wait	†Days	2.81	4.32	$.650 \pm .045$	Bryan & Beard (44)
Phenol, cresols	Goldfish	Death	†Log-minutes	.297	1.906	$.156 \pm .011$	Gersdorff (122)
o-, *m*-tolyl mercaptan	Goldfish	Death	†Log-minutes	.105	.948	$.111 \pm .006$	Gersdorff (123)
p-tolyl mercaptan	Goldfish	Death	†Log-minutes	.105	.670	$.157 \pm .012$	Gersdorff (123)
Sodium arsenate	Silkworm larva	Death	†Log-minutes	.100	.493	.203	Bliss (26)

* Prothrombin time.
† These have a negative slope.

onic chick heart in solutions of known concentrations to the first appearance of atrioventricular block, and Lehman & Paff (178) have adapted this technique to the assay of digitalis. Jaques (159) has described a similar quantitative assay for thrombin and prothrombin based upon clotting time. In both cases linear dosage-response curves were obtained by plotting the logarithm of reaction time against the logarithm of dose of drug.

The inherent precision of several techniques based upon reaction time has been computed in terms of the ratio of the standard deviation of response to the slope and listed in Table II (pg. 498). To obtain equally precise estimates of relative potency from two procedures that differ in this ratio, the number of observations on each assay must be inversely proportional to λ^2 (33).

Assays Based Upon a Graded Response

In this class of biological assays the extent of the reaction is a function of the dose of drug. Ideally, the quantitative relation between dosage and response would be expressed in terms of an equation describing the mode of drug action. Clark (60) has shown that many reactions in this category are consistent with the mass-action formula, in which the percentage action may be plotted as a symmetrical sigmoid curve against the logarithm of the dose. Emmens (93) has fitted with marked success another sigmoid curve, the simple logistic, to the gain in weight of various internal organs under graded doses of the appropriate hormone. When they can be tested over a wide enough range of doses, most graded reactions are consistent with the sigmoid form, approaching asymptotically to a "floor" and a "ceiling." However, unlike the all-or-none response, where the number exposed to treatment is known or can be estimated readily from control groups, the amount of reaction representing 100 per cent of a graded response would need to be determined experimentally to solve either formula. Since this is practicable in relatively few assays, most procedures have avoided the ends of the curve and based their estimates of potency upon its linear central portion. In terms of the log-dose this has covered a wide enough range for a majority of assays.

Graded responses may be measured by a wide variety of criteria, and even a single procedure has offered a choice between two or more alternatives. Biological variables have often been "cor-

rected" from assumptions as to the mode of drug action, which have complicated some assays unnecessarily or even reduced their potential value. For selecting the best criterion of response or the best procedure for minimizing variability, certain conditions have been proposed by Gaddum (114) as a basis for action. These may be summarized as (*a*) a criterion of response which will plot linearly against the log-dose over the widest practicable dosage range, (*b*) a standard deviation in response (*s*) which is independent of dose, and (*c*) a minimal value for the standard deviation of dose or the ratio $s/b = \lambda$.

Since most assays based upon either reaction time or a graded effect are carried out at dosage levels where the standard deviation is constant, weighting coefficients, such as are used with a quantal response, are not needed. Hence they can be computed with relatively simple applications of the analysis of variance if properly designed. Because of their importance in balancing potential errors, these newer designs will be considered more fully under a later heading. The statistics of assays based upon a graded response have been discussed generally by Irwin (155), and by Bliss & Marks (33), Morrell (210), and Schild (230) with reference to tests meeting certain minimal requirements, which simplify the calculation. These include the same number of separately recorded reactions on each dose, two or more doses of both standard and unknown spaced equally on a logarithmic scale and the balanced allotment of test material between doses so that one dose is not represented by a potentially more susceptible reaction series than another. An assay is then self-contained. Comparison with the experimental error tests the divergence from parallelism of the log (dose)-response curves for standard and unknown and if three or more doses have been used, the significance of curvature from the two straight lines. If the assay meets these tests of its validity, one can estimate the combined slope of the two log (dose)-response curves and the relative potency of the unknown with its error. Alternative designs and methods of analysis will be discussed with the different types of graded response.

Assays based upon a growth response.—Biological assays have been based upon (*a*) the growth of the animal as a whole in terms of its live weight, (*b*) the growth of an organ or tissue affected directly by the drug, (*c*) the relative growth of some constituent in the body, and (*d*) the selective growth response of microorganisms.

(*a*) The growth of the intact animal is a general criterion af-

fected by many materials so that supplementary evidence may be required as to the presence of deficiency symptoms characteristic of the factor under test, as in the U.S.P. assay for vitamin A. Coward (64) and others (176, 199) have shown that the gain in weight of depleted rats is a linear function of the log-dose of vitamins A and B_1.

For vitamin A, the standard deviation for gain in weight is independent of the dose, is larger for males than for females, and increases as the assay period is lengthened. The slope of the dosage-response curve, however, is enough steeper for males than for females to give assays of vitamins A with male rats a greater inherent precision than those with females (64). The slope increases more rapidly than the standard deviation as the assay period is lengthened from one to eight weeks (64, 249), but the resulting increase in precision after three or four weeks on the test diet probably does not justify the cost of prolonging the assay (10). One recommendation of a five instead of an eight week period (249) was based erroneously only upon the standard deviation of the response (13.9 as compared with 18.1). Reanalysis of the same data showed λ to be 0.28 and 0.20, respectively. Although the gain during the first week on the test-diet may be relatively irregular, Coward (63) found its omission would not improve the assay of vitamin A in her own laboratory.

In the growth assay for vitamin B_1, λ is less than half as great as that for vitamin A in Coward's laboratory (63); this fact permits an assay period of three or two weeks. Although others have used periods up to five weeks, the precision did not increase from three to five weeks; the values for λ were 0.20 and 0.21, respectively (199). Sherwood & Halverson (236) found similarly no improvement beyond three weeks but low precision with a seven-day period. Two papers, however, have described three- and four-day single-dose assays, in which the same animals could be tested several times (161, 210). Alternatively several workers (176) have administered a single dose of vitamin B_1 to the depleted rat and computed an "index" of vitamin activity as the product of the maximum gain in weight, multiplied by the days to return from this maximum to the initial weight. Unlike the vitamin-A assay, Coward (64) found no consistent difference between males and females either in the slope of their dosage-response curves or in the standard deviation of the gain in weight.

Growth methods have also been reported for riboflavin or

vitamin B_2 (89, 94). In the central range of doses (6 to 32 μg.) the gain in weight of the rat over four weeks could be considered a linear function of the log-dose although sigmoid with a wider range. The adrenal cortical hormone has been assayed from the growth of adrenalectomized rats (130) and the growth hormone of the anterior pituitary body from the weight increase of hypophysectomized rats (47, 197) as the curve of gain-in-weight against log-dose is similarly linear at intermediate doses in both cases. There is finally, of course, an extensive literature on the bioassay of various plant nutrients and hormones based on the growth of plants, but this lies outside the scope of the present article.

(*b*) Where a drug, usually a hormone, acts upon a specific organ or tissue, its activity has been assayed from the increase in size of the affected part. Some investigators have applied the drug locally, as in the cock's comb assay of the male sex hormone from changes in comb volume (95) or that of the growth hormone, auxin, from the angular deflection of root tips (251). More commonly, however, the drug has been given systemically by subcutaneous or intraperitoneal injection and the response measured in terms of the size of the organ, usually its weight after extirpation. Boyland (37), however, has measured *in situ* the length and width of mouse tumors repeatedly with calipers to determine the effect of various aldehydes and their esters upon tumor growth; his criterion was the ratio of the growth rate in the treated period to that in the preceding control period.

One of the more troublesome factors in using the weight of an organ for an assay criterion is the frequent dependence between the mean weight and the standard deviation. In the assay of prolactin, for example, the weight of the crop gland of the dove or pigeon proved a linear function of log-dose over a suitable range of doses (17) but the standard deviation of the weight was also a function of the dose. In a dosage-response curve for uterine weight of the rat following different dosages of estradiol, Eisenhart (88) found that the square root of the weight was related linearly to the log-dose with a uniform standard deviation at five dosage levels. Baker (14) has quoted data on the weight of rat ovaries at five different dosage levels of gonadotrophic hormone where reanalysis disclosed a linear relation between the logarithm of the weight and the log-dose. In terms of logarithms, the standard deviation in the response was relatively independent of the dose. A similar linear

relation between log-weight of an organ and the log dose of drug has been observed in other assays (22, 180, 235).

Frequently, however, the weight of the organ plots as a straight line against log-dose with the standard deviation of the weight also a function of the dose (93, 96). Koch (166) has furnished a series of 151 individual weights of the mouse ovary covering a more than one-hundred-fold dosage range of gonadotrophic hormone. Here the mean weight is a linear function of the log-dose and the standard deviation of the log-weight is independent of dose. In consequence, the variance within doses is proportional to the square of the expected response (Y^2). In the assay of gonadotrophins from several sources, Emmens (93) has reported that the variance in the ovarian weight of the immature rat is equal to 0.10 Y^2, and in the assay of prolactin, he has found the variance in the weight of the pigeon crop gland to be 0.12 Y^2. In such cases the information in each average response is inversely proportional to the square of its expected value, and the calculation resembles that of quantal assays with probits and log-doses. In the experiments reported in Table III, both organ weight and its logarithm were related linearly to the log-dose of hormone with about equal frequency, so far as could be judged from the available data. It seems probable that a critical study of more detailed data from several laboratories would reveal a biological explanation for the apparent discrepancy or show one of these relations to hold within much narrower limits than the other.

Some hormone preparations have been assayed from both uterine and ovarian weights with differing opinions as to their relative merits. Graded doses of chorionic gonadotrophin, for example, lead to a rapid increase in uterine weight which may then decrease slightly to a plateau, while ovarian weight increases more slowly but steadily over a much wider range of doses. Largely because of the lower range of effective dose some have preferred uterine weights (15), but Delfs (79) has warned that it must not be accompanied by marked ovarian stimulation to insure against exceeding the useful dosage range. Others have found the ovary to give more precise results despite its lower sensitivity, with the advantage that it is a specific indicator which the uterus is not (22, 61). In his analysis of the collaborative assay on the international standard for the gonadotrophic substance of human urine of pregnancy, Emmens (92) found the variance for uterine weight to be over

twice that for ovarian weight so that the latter was much the more sensitive.

Graded responses based upon the growth of specific organs include gain in weight of the thyroid gland (106) and increase in thyroid cell height (222) in chicks following treatment with the thyrotrophic hormone of the anterior pituitary, increased weight of the uterus of the immature rabbit with estrone (193), and percentage reduction in the length of the tadpole tail when treated externally with thyroid powder (269); in all cases the response was a linear function of the log-dose. Schultzer (233) has described an assay for vitamin D with rats based upon the direct measurement of the width of the metaphysis, which is related linearly to log-dose. This latter technique is more properly, perhaps, a simple numerical variant of the bone ash or line test for vitamin D.

(*c*) Relative growth of a bodily constituent has been used most prominently in the assay of vitamin D in terms of the bone ash, primarily calcium phosphate. This has been expressed most frequently as the percentage of ash in the dry, fat-extracted tibia or femur. In a statistical study of a rat assay (31), percentage of ash proved a less efficient criterion than the log-weight of ash; both were related linearly to the log-dose of vitamin D. The linear relation between individual ash weights and bone weights and its zero intercept led Knudsen & Tolle (165) to conclude that in the chick assay for vitamin D, the percentage of ash was a suitable criterion. Since twelve to twenty chicks are commonly caged together and given a single dose mixed with the food, the error of the assay can be judged adequately only from the results of replicated cages, not from the variation between individuals within cages. The average of the individual percentages of bone ash proved no more satisfactory a criterion than the ratio of the total ash to the total weight of bone for the group so that in routine assays pooled values are sufficient (186). The discrimination of different doses of vitamin D increased with the length of the chick assay but a five-day depletion diet prior to the test period reduced the slope of the dosage-response curve. Female chicks seemed a little more sensitive than males to changes in vitamin D and seasonal influences were marked (170).

A method has been described by Campbell (52) for assaying the potency of certain anterior pituitary principles from the liver fat in mice. The milligrams of fat increased linearly with the log-dose of extract. The slope of the dosage-response curve was steeper.

in female mice, but due to the difference in the standard deviation the assay was more precise in males ($\lambda = 0.22$ and 0.34 for males and females, respectively). Because of the more uniform increase in liver fat over a seven-hour than a sixteen-hour period, the former was recommended, in which three mice gave as much information on potency as five mice.

(*d*) Microbiological assays depend upon the selective growth response of different strains of microorganisms, usually yeasts, to specific vitamins and related compounds. Response may be measured in terms of gas production, turbidity, weight of material produced, and in other ways. This field is so extensive that it could not be included within the limits of the present article.

Responses involving both intensity and duration.—In most graded-response assays, a change in dose modified both the intensity and the duration of the reaction. This is seen most clearly, perhaps, in the changing blood sugar of the rabbit following subcutaneous injection of insulin. Periodic blood samples, covering an interval from just prior to treatment to five or eight hours later, show that the larger the dose of insulin the greater is the fall in sugar and the more delayed the return to normal. The response to many other drugs follows a similar course, with the action positive in some cases and negative in others. Various characteristics of the complete curve have been measured as the criterion for biological assays. These include (*a*) the area enclosed by the base line and the curve or its equivalent, (*b*) the maximum (or minimum) height of the curve, (*c*) the height at some fixed interval after treatment, and (*d*) the time for the response to reach a maximum or half completion. In some cases the choice among these alternatives is limited by the nature of the assay but in others has probably been due to convention.

(*a*) The assay of insulin has used a larger part of the curve than that of most other drugs. Kohl (167) plotted a blood sugar curve based upon nine periodic samples for each dose and determined the area under the curve in square centimeters for his criterion. Others have used five hourly readings (195) or three readings at one and one-half, three, and five-hour intervals (169); but for a given bleeding schedule, the mean fall in blood sugar is still essentially an integration of the blood-sugar curve even though based upon a smaller number of observations. Some workers have pooled the successive blood samples on each rabbit (195), which would preclude their differential weighting if they were of unequal value for

discriminating between doses of insulin. Others have pooled the samples from all rabbits of a group for each period, a less acceptable short-cut since it is then impossible to compute the intrinsic precision of each test. The mean fall in blood sugar is usually expressed as a percentage of the initial level although it has been shown that this arbitrary correction is inadequate and needs further adjustment (33, 98, 142). The percentage fall in blood sugar has proved a linear function of the log-dose (33, 99) so that relative potencies computed from the difference in the response on the standard and on the unknown have been found to have the smallest error (99). Many workers, however, have preferred to keep within a narrow range of doses and base their estimates of potency upon the ratio of the mean responses on the two preparations (169).

An assay procedure for diuretics has been described (181) which integrates the area under the curve experimentally. Fasting rats were given doses of drug dissolved in a volume of warm isotonic salt solution equal to 25 cc. per kg. and their urine collected over a period of five hours. By this time a marked decrease in the rate of excretion indicated that the effect of most drugs had disappeared. The log-ratio of the volume of excretion to that in a saline control was used as a measure of diuretic action and related linearly to the log-dose of drug. Certain drugs required a twenty-four-hour test period and showed a decreased effectiveness as the dose increased past a value giving maximal diuresis. Unfortunately at each dose the excretion from two four-rat groups has been pooled by the authors so that they lack a good estimate of the standard deviation of diuretic effect.

An antidiuretic assay of pitressin has been described (160) which similarly integrated the area under part or all of the curve. The bladders of individual rats were cannalated so that urine could be collected continuously; the measure of antidiuretic action was the difference between the volume secreted during the twenty minutes after injection of the drug and that secreted in an equivalent period immediately before injection. As many as four injections could be tested in a single rat, and to the extent that the antidiuretic action was completed within a twenty-minute interval the assay integrated the curve of response. The response was a linear function of the log-dose but the authors mistakenly have reversed their true independent and dependent variables in computing the dosage-response curve.

(*b*) Frequently the duration of response is neglected in favor of the maximal reaction, as in the U.S.P. assay for epinephrine or for the oxytocic principle of pituitary extract. With these substances the kymograph drum may be stopped giving a record only of the maximal excursion of the lever. Since the response on the standard is usually matched against that on the unknown, relatively few dosage-response curves have been reported for assays of this type. Dosage-response data for epinephrine and other sympathicomimetic substances have been plotted on different coordinates by Hjort *et al.* (149), who found a linear relation between the logarithm of the pressor response in millimeters of mercury and the log-dose of drug. They note that unless the log(dose)-response curves for two drugs or samples are parallel, which would indicate qualitative equivalence, relative potencies obtained by matching blood pressures may be misleading.

The diameter of the pupil of the mouse has been measured periodically after subcutaneous injection of extracts of belladonna and stramonium and the maximum diameter expressed as the ratio to the diameter before treatment by Watson *et al.* (263). The average pupil ratios were related linearly to the log-dose and gave biological estimates of potency higher than check values obtained chemically, a discrepancy due apparently to the presence of active principles missed in the chemical analysis. The maximal dilation of the pupil of the rabbit eye has been used similarly in the assay of ergonovine by deBeer & Tullar (75), who found the maximal value more satisfactory than that at any given interval after treatment. Parallel measurements on rectal temperatures provided an alternative or accessory criterion for the quantitative estimation of the drug.

Experiments by Coon (62) measured the blood pressure in the domestic fowl under sodium phenobarbital anesthesia when injected with posterior pituitary extracts. Within a limited dosage range the fall in blood pressure was proportionate to the dose but the sensitivity decreased in successive tests on a given animal, which usually was good for assaying six or more preparations. Schild (230) has described a quantitative assay for histamine from the contractions of isolated gut of the guinea pig. His procedure gave an estimate of relative potency and its precision from each section of gut, based upon its own linear relation between log-dose and response. Bias due to the decreasing sensitivity of the preparation was avoided by the use of successive replicates, each con-

sisting of two doses of standard and two doses of unknown spaced equally on a logarithmic scale and given in a random order.

(*c*) For a practicable assay the character of the response may limit its measurement to one or two fixed intervals after treatment. In assaying insulin from the blood sugar of hypophysectomized and adrenodemedullated rats, where the small size of the animal would preclude the use of many samples, Gellhorn *et al.* (121) have relied upon a single blood sample taken sixty minutes after injection. The chemical determination of calcium is so time-consuming that parathyroid assays are usually based upon a single post-treatment value whether from a sixteen- to eighteen-hour serum sample in dogs (U.S.P. XII) or the twenty-four-hour urinary calcium output in rats (253), both giving linear log(dose)-response curves. Estrogens have been assayed from the hydration of the rat uterus as measured by the increase in uterine weight six hours after treatment (9) with only a single observation being possible due to the nature of the response.

In other cases a single measurement has been more convenient and considered by the author as sufficiently sensitive for bioassay purposes. Boyd & Mack (36) have determined the percentage gain in weight of frogs over an eighteen-hour period due to uptake of water after injections of different doses of pituitrin. Coal-tar antipyretics were tested against artificial fevers produced in cats by injection of *E. coli* vaccine and in rats by a suspension of yeast acacia solution (42). Rectal temperatures were recorded before and for three successive hours after injection of the antipyretic; the fall in temperature in rats plotted linearly against the log-dose. Testicular extracts have been compared (12) from the area of blebs appearing on the shaven backs of rabbits and measured twenty minutes after the intradermal injection of three doses of the standard and three doses of unknown. As a fourth example we may cite the assay of liver extracts from the migration of cells from isolated bone marrow of the guinea pig (117, 216). Pieces of marrow were transferred to Stender dishes, covered by diluted plasma containing differing amounts of liver extract, and as soon as the plasma had set, the dishes incubated at 37.5° for fifteen hours. The movement of cells into the coagulated plasma was projected on to paper for measurement of their area and the migration expressed as a percentage of the controls for plotting against the dose of extract.

(*d*) Instead of determining the response at a uniform time after treatment, the time to reach a fixed stage in the response has been used for some biological assays. This differs from reaction time *per se* in that the stage selected as the end-point differs quantitatively but not qualitatively from the preceding phase of the reaction although the two types grade into one another. The technique has been used for the assay of diuretic action in rats from the time elapsing between injection of the drug and the maximal rate of excretion (49, 110). By reading the volume of urine at fifteen-minute intervals, the time for maximal excretion could be interpolated for preparations of pituitary extract. Experiments by Morrell & Bliss as yet unpublished indicate a linear relation between the logarithm of the median excretion time and the log-dose of pituitary extract.

Response scored in nonnumerical grades.—In a number of assays, reactions which change progressively with the dose of drug may be difficult to measure directly so that the experimenter assigns grades on an arbitrary scale to the different degrees of response. The average score for all individuals on a given dose then comprises one type of graded response.

The relation between scores and alternative techniques is illustrated in the assay of estrogenic preparations from the incidence of cornified cells in vaginal smears. Smears may be classified arbitrarily into the two categories of positive and negative and assays based upon the percentage of "positive" individuals at each dose. Many investigators have preferred this "quantal" reaction for the assay of gonadotrophic substances although Burn (49) has shown how the stage designated as "positive" varies from one worker to another. Hemmingsen & Nielsen (143) have classified these smears on the basis of their histological composition into six grades from 0 found in untreated ovariectomized rats to 5 in full estrus. The mean scores permitted assays of solutions containing from 0.2 to 6.0 "rat units." Classification into grades, however, depends largely upon the percentage of leucocytes or of squamous cells. With the differential stains now available (237), arbitrary grades could be replaced with a count in each individual of the proportion of a given type of cell for a fully objective graded response.

Other scoring techniques have been proposed for various hormones. Frank & Berman (107) have assigned "ovarian values"

from 1 to 9 to various degrees of follicle growth and luteinization in serially sectioned ovaries of immature rats, which they preferred to several alternatives for the assay of gonadotrophic factors. With a mucification test on mice, recognizing six grades of reaction from transverse histological sections of the vagina, Deckert *et al.* (76) reported more reliable assays of estrogens in human blood than with the vaginal smear technique. Two papers (171, 221) have described assays for hormones of the posterior pituitary from scores on the expansion of the melanophores in hypophysectomized lizards and frogs. The average score in lizards was a linear function of the log-dose.

Reactions scored in nonnumerical grades have also been used in vitamin assays. Assays for vitamin A based upon the severity of ophthalmia (260) or of medullary lesions (154) have not yet been developed into quantitative tests. The line test for vitamin D, on the other hand, has been used quantitatively. It is based upon the degree of calcification in a depleted rat of a leg bone split lengthwise (U.S.P. XII) or in that of a chick in cross section (190) but a uniform scoring system has not yet been adopted by all workers (225). Although O'Brien & Morgareidge (214) related degree of healing linearly to dose, Coward & Kassner (66) found a linear relation between degree of healing and the log-dose both of phosphorus and of vitamin D.

Scores have been avoided altogether in Schutz's (234) assay for anticoagulants. Dilution series were prepared for the standard heparin and for an unknown each in a rack of twelve tubes with the successive concentrations in the same geometric progression. Blood directly from the carotid of an anesthetized rabbit was added to the two series alternately, mixed and held at a constant temperature until the next day. The tubes formed two "dosage-response curves" in terms of coagulation and sedimentation which could be superimposed; the relative concentrations of standard and unknown in corresponding tubes determined their relative potency.

The human assay of digitalis described by Gold *et al.* (125) has been based upon the relatively constant reaction of the T-wave and the RT or ST segments of the electrocardiogram to digitalis in the same patients. Individuals were calibrated at one-month intervals with three doses of standard digitalis differing by 22 per cent and with only those used in assays whose tracings differentiated consistently between doses. An electrocardiogram was taken after a single

dose of unknown and the four tracings were again ranked in order of digitalis effect for each individual separately; the potency of the unknown was determined from its average position relative to the three doses of standard.

Instead of ranking the effect without distinction as to the extent of the difference between successive items, the grades assigned visually to different degrees of response may be converted to numerical values which will best differentiate between doses of drug. A grade of 0 is assigned to the completely negative condition and 1 to that representing maximal response. Fisher (101) has described how scores may be determined for the intermediate grades which are additive and will best differentiate between treatments. In a series of serological readings, for example, scores of 0, 0.10, 0.58, 0.96 and 1.0 replaced five arbitrary grades, but with 144 readings were not significantly better.

Changes in Experimental Technique for Increasing Precision

With a view to reducing the variables inherent in all assay methods, experimental techniques have received much attention. These include greater purification of preparations and the separation of the active constituents into different components, which improve the inherent ability of a bioassay to discriminate differences in potency, but such methods are outside the scope of the present discussion. With greater knowledge and control of the factors contributing to variability in response, the use of a reference standard becomes correspondingly less essential. A more important result is the reduction in the error of individual assays, with the consequent possibility of obtaining satisfactory estimates of potency with fewer animals. Many studies for improving assay technique, however, have been concerned primarily with reducing the dose of drug which will evoke a measurable response. Although the heightened sensitivity may provide assays for smaller quantities of material—often a factor of importance—this change in the threshold of response may be quite independent of the precision of the assay. In a number of experiments to be cited, the relative precision of the more sensitive techniques has not been examined critically. The following modifications in method have proved useful.

Increased homogeneity of the test animals.—Bioassay techniques

have profited by the contribution of the geneticists and it is now customary, in carrying out assays involving numbers of small animals as in testing vitamins or hormones, to employ an inbred strain. A further refinement, discussed on page 520, is the use of litter mates.

In many assays, particularly those utilizing immature animals, greater uniformity or sensitiveness has been achieved by restriction to certain ages or limiting differences in age. For estrogens assayed on ovariectomized rats by the induction of estrus the sensitivity of the animals has been found to increase up to the age of six months (18) whereas the sensitivity of mice appears not to vary with age (18, 91). More uniform growth following local application of male sex hormones was observed with three-day old chicks than with six-day old chicks (108). In the assay of the growth-promoting hormone of the anterior pituitary gland in rats, older and heavier animals proved less responsive (197). Moon (207) has utilized four-day old rats for the assay of the adrenocorticotrophic hormone of the pituitary and obtained graded increases in the weight of the adrenal gland in relation to dose, with about twice the sensitivity found in earlier studies with twenty-one to twenty-three-day old rats. These examples illustrate the importance of selecting the optimal age in the assay of hormones.

In a number of other drugs, however, there is evidence that age and body weight are of little importance in potency determinations when expressed on a per kilo basis. To cite a single example, Haag & Corbell (132) compared the susceptibility of kittens (average weight, 0.75 kg.) and full-grown cats (average weight, 2.7 kg.) to a number of digitalis preparations by the continuous infusion method of Hatcher & Brody. With twenty animals for each preparation of digitalis, no significant difference was found in the susceptibility per unit of body weight of kittens and cats.

In hormone assays which utilize sex characteristics a single sex is necessarily employed, but in certain others this restriction has increased the uniformity of response. In the use of day-old chicks for the assay of androgens by local application to the comb, Emmens (91) noted a steeper log(dose)-response curve in the males but a smaller variation in response in the females. In androgen assays with adult birds the use of hens obviated the need of castration and appeared to be more satisfactory (69). Thyrotrophic hormone assayed by the increase in thyroid weight in day old chicks

(20) showed slopes twice as great for the males as for the females; this fact indicated a greater sensitivity to the hormone in the male. Coward (64) has shown that male and female test animals differ in the slope of their log(dose)-response curves for vitamin A so that the sexes should be computed separately. In vitamin-D assays, day-old chicks of the same sex have been recommended, usually males (53). Squill in rats affords a striking example of a sex difference in susceptibility since it is approximately twice as toxic to females as to males (187).

Better preparation of the animal for assay.—A variety of procedures serve to increase the responsiveness of the assay animal to the drug. In the assay of liver extracts the search for a suitable test object to replace the inconvenient and inexact reticulocyte response in the pernicious anemia patient has developed various methods for producing anemia in animals. These include the injection in the rabbit of a saponin-collargol mixture (128, 226, 229, 274), hydroxylamine (229), fatty acids (274), and subacetate (185), all of which produce anemias responsive to liver extracts. In the rat a hypochromic anemia has been produced by removal of the spleen in animals infected with *Bartonella* (226), by injection of a saponin-collargol mixture (274), and by the injection of a suspension of glycocholic acid from ox bile (158). Without such treatment the reticulocyte response in normal animals, including the guinea pig (224), the pigeon (261), and rat embryos (172), has lacked sensitivity.

Most vitamin assays involve the use of deficiency diets of which various modifications have been proposed. Their composition is not germain to the present discussion, but a few examples will illustrate the importance of these conditioning diets. Placing the mother on a vitamin A-free diet from the time of conception has improved the sensitivity of the growth response in the rat (10, 127). In vitamin-B_1 assays Nelson & Kline (176) have given the deficient diet to the mother during the nursing period.

Modification of the basal diet to make it more complete for other factors and by this means to produce an uncomplicated vitamin-B_1 deficiency syndrome has improved the accuracy of the assay and permitted the use of the same animal for repeated curative tests (164). Better results have been reported when autoclaved whey was substituted for yeast (231); others recommend the use of sulfite for the destruction of vitamin B_1 in the basal diet (163).

In providing adequate supplies of vitamins A, B_1, and C, amounts in excess of an adequate dose apparently had no influence upon the assay of vitamin D by the line test in rats, and an excess of vitamin D did not affect the assay of vitamin A (43).

In a study of various factors influencing the assay of red squill a high protein, low carbohydrate diet has been found to decrease the toxicity (187).

Inanition diets have proved useful in improving the sensitivity of the response in certain hormone assays. By reducing the food intake Breneman (39) inactivated the hypophysis, which interferes in the assay of androgens by the chick comb method, and by this means he enhanced the sensitivity of the assay. Similarly, Stephens (246) has reported that undernourished guinea pigs show a marked increase in sensitivity to the thyrotrophic hormone. The thyroid of such animals may be stimulated by the injection of as little as one tenth the minimal effective dose for normal animals.

In digitalis assays by the Hatcher-Brody technique the anesthetic influences the sensitivity. In general, animals require about 25 per cent more digitalis when nonvolatile anesthetics are used as compared with light ether anesthesia. This has been shown for urethane (86), for chlorbutanol (135), and for seconal and amytal (113).

Another procedure that has improved the sensitivity in certain hormone assays has been pretreatment with a conditioning dose of the same or other substances. In Palmer's method (217) for the assay of the estrogens in castrated female mice, an overdose of the estrogen is followed by small doses at regular intervals, which keeps the mice constantly reactive to a minimal amount. Emmens (91) gives a priming dose only when the rat or mouse has not been used in an assay for several weeks or has failed to respond in a previous assay, or to improve the sensitivity of the animals some weeks before the assay. Estrone also increases sensitivity to the mammogenic factor (205). Progesterone is assayed from the response of the uterus in the immature female rat (137) or rabbit (58) following pretreatment with six daily doses of estrogen. In cats a modified procedure has been recommended (256) in which the uterus is sensitized by two doses of estradiol on successive days followed by three daily doses of progesterone. Thirty hours after the last injection, the effect of epinephrine and hypogastric stimulation on the contraction of the uterus is recorded as an all-or-none response.

Pretreatment of rabbits with estrogenic substances has been found to increase the percentage of uteri responding in the assay of ergonovine (267). The addition of palmitic acid to the solvent reduced the ED50 for estrone in ovariectomized mice to less than half its value in oil alone, but at the same time gave a flatter log(dose)-response curve and a more difficult solution to handle (91). Here a change in technique which increased the susceptibility to the drug actually reduced the precision of the assay.

In many hormone assays the appropriate organ is removed to produce a deficiency which affords a more sensitive base for observing the response. In the case of the sex hormones, spayed or castrated animals are usually employed or, alternately, immature animals or those of the opposite sex. Adrenalectomized and hypophysectomized animals showing a markedly increased sensitivity to insulin have been studied with reference to their possible value in insulin assay (121, 139, 144). A four to tenfold increase in sensitivity may be obtained so that minute amounts of insulin in body fluids can be detected. However, in experiments with adrenalectomized rats and mice Hemmingsen *et al.* (144) found the slope of the log(dose)-convulsion-rate curve was about the same as in normal animals, and they, therefore, doubt that percentage accuracy can be increased by this method.

Extracts of the adrenal cortical hormone are assayed upon adrenalectomized animals, usually upon dogs. A satisfactory quantitative method with thirty-day old adrenalectomized rats has been described by Grollman (130) following Schultzer (232), but using growth rather than survival.

Changes in the conduct of the assay.—To minimize as many sources of variation as possible, laboratory procedures have been standardized in greater or lesser degree for each of the various bioassays. Certain aspects which are common to many of them may be reviewed briefly. These are (*a*) the route of administration and (*b*) the treatment schedule, both of which may have an important influence on the assay and have been the subject of numerous investigations.

(*a*) In androgen assays there has been much discussion but no agreement as to the best route by which to administer the hormone. The capon and immature birds respond to much smaller doses of androgens when applied locally. Some have preferred to apply the hormone directly to the surface of the comb (91, 109),

while others have recommended injection into the comb to eliminate the possibility of poor absorption (39).

Intravaginal application has been found a sensitive method for detecting the presence of estrogens. Although suggested for a quantitative method in rats (3), in mice it proved to be unsatisfactory (91). Intercutaneous injection of prolactin over the crop sac in pigeons offers a sensitive method for its detection, and only one one-thousandth or less of the systemic unit is required, and there is the added advantage of greater specificity (17, 198). Following pretreatment with estrone, the uterus of the immature rabbit has been found to respond by endometrial proliferation to the direct injection of small doses of progesterone (136, 192). The variability of the reaction limits its use quantitatively, but as a qualitative test it is more sensitive than intramuscular injection. In rats and mice little difference has been noted in the response to gonadotrophic material by intravenous or subcutaneous injection (134).

The route of administration has had an important bearing upon differences in the assayed potencies of the cardiac drugs. The one-hour assay for digitalis in the frog showed a significantly faster absorption from the pectoral than from the abdominal sac (204) and a significantly slower absorption rate from the lymph sac than from intramuscular injection (203). Great differences in absorbability have been observed between the various active constituents of the leaf and among different purified digitalis glycosides (252, 257, 271). These are reflected in the discrepancies in potency of digitalis preparations when given intravenously as compared with oral dosage so that the cat method in the U.S.P. XII may not give the therapeutic potency of orally administered preparations (124).

(*b*) Many dosage plans have been employed in the assay of the vitamins and hormones and a number of investigations have concerned the most suitable schedule. The greater healing on a divided than on a single dose of vitamin D reported by Bacharach *et al.* (11) as compared with the equal effectiveness observed by Coward & Key (67) has been studied by Edwards (87) and explained in terms of duration of the test and the severity of the basic ration. The effect upon precision of the duration of vitamin assays for given rates of treatment has already been noted.

Gonadotrophic hormones have been more effective on some than on other dosage schedules. In assays by the vaginal smear

method, ovariectomized rats have shown a lower susceptibility to estrone when it is given either in a single dose or in twelve injections at hourly intervals than as morning and evening injections over a two-day period (91, 133). In experiments with one hundred day old mice the same amounts of estrone gave a steeper dosage-response curve in terms of ovarian weight when administered in ten than in four portions (22). In a study with gonadotrophic hormones in rats, the response was more uniform when the single dose was divided into three daily portions (138). For the hormone of human pregnancy urine a single subcutaneous injection in immature rats and mice is reported to be only one fourth and a single intravenous injection one eighth as effective as when divided into five portions (134).

Another aspect of the problem relates to the interval between giving the dose of drug and recording the results. The frog assay for digitalis has been studied with respect to the comparative reliability of the one- and eighteen-hour methods (55, 153, 201, 203). There is a general agreement that by extending the time to eighteen hours and using fatal effect rather than systolic standstill as the criterion the reliability of the assay is improved.

Assays against an antagonist.—A number of assay techniques have been proposed which involve antagonism to some other active drug. The dorsal muscle of the leech, which has been used extensively for the estimation of small quantities of acetylcholine, has been suggested (70) for the assay of various alkaloids from their effect on the contraction produced by acetylcholine; morphine, codeine, prostigmine, and quinine augment and strychnine and caffeine reduce the response. For the assay of morphine the antagonism of acetycholine hydrobromide has been useful for measuring very small quantities (97). Renin restores the blood pressure in dogs after it has been depressed by a known dose of yohimbine and this has been proposed as an assay method (112).

Further examples of standardization against an antagonist are to be found among the hormone assays. For the assay of parathyroid extracts, Dyer (84) has proposed the antagonism of the narcotic effect of magnesium sulfate in mice. Following injection with the test extract the magnesium salt is administered and potency determined from the proportion of mice unable to turn over when placed on their backs. In guinea pigs the action of follicular hormones in inciting estrus in immature animals has been prevented

by the daily injection of small quantities of progesterone, and this is reported to be more sensitive than other methods (150). Szarka (250) has described a quantitative assay of progesterone based on the inhibition of estrus produced by the injection of estrone in ovariectomized adult and in immature female rats.

Increased Precision Through the Design and Analysis of the Assay

Despite improvements in experimental technique, the elimination of some sources of variation is either impossible or impracticable. Many of these can be balanced in the design or adjusted in the calculation so that they will not interfere with the estimate of relative potency or inflate its computed error. Though applicable to all-or-none assays, the relative importance of these safeguards in design can be assessed more readily in assays based upon reaction time or a graded response.

The appropriate technique depends upon whether the factors contributing to the error of an assay are qualitative or quantitative (33). Qualitative factors lack a numerical characteristic from which their effect upon the response can be determined. They include differences in susceptibility between individuals, litters, and days and must be balanced in setting up the assay. The effect of quantitative factors upon the criterion of response can be described by a relatively simple curve. These include differences in age, weight, and the initial level of the measure of response, which can be allotted at random to the different treatments and adjusted later by calculation. Both types may occur in a single assay as in the U.S.P. XII assay for vitamin A where six factors are balanced in assembling test groups but without quantitative evaluation of the result.

Apart from these improvements in design and analysis, the rejection of aberrant values has sometimes been proposed for increasing precision. Finally there are the techniques for utilizing past experience in assays made repeatedly in the same laboratory.

Balancing qualitative sources of error.—Among qualitative sources of variation, that between individuals has contributed largely to the error of biological assays. Following Clark (59), this may be divided into two components, static variation representing persistent differences in susceptibility between the individuals of a group, and dynamic variation in the susceptibility of one individ-

ual relative to another from test to test. To the extent that static exceeds dynamic variation, assays gain precision when more than one response can be obtained from each animal and they are based upon comparisons within rather than between individuals. Since the susceptibility of test animals tends to vary as a group, possible trends in overall susceptibility from one observation to the next on the same animals have been balanced in the cross-over tests. At the first treatment half of the animals, chosen at random, are given the standard drug and the other half the unknown, and at the second treatment the materials are reversed so that each animal receives both.

The cross-over test was first introduced by Marks in the rabbit assay for insulin where it is now used exclusively. In two experiments, for example, segregation of individual variation in susceptibility improved precision to the same extent as more than doubling the number of observations (33, 98). Since the cross-over test includes only one dose of both standard and unknown, the slope of the log(dose)-response curve has been estimated from separate tests on the same sample of unknown run at two or more doses (98, 99). Brandt (38) has described designs for testing two groups of animals two, three, and four times in reversal or switch-back trials. The usual cross-over test is in effect a 2×2 Latin square which has been extended to the 4×4 Latin square for the dual segregation of differences in individual susceptibility and of day to day variations by Bliss & Marks (33). Since the 4×4 Latin square tested two doses of standard and two doses of unknown on each rabbit in the group, an estimate of the slope of the dosage-response curve formed an integral part of the assay. Balanced experiments requiring more than two consecutive tests on each animal may be disturbed by the death of one or more individuals before the test is completed (169), but simple methods for computing the results of incomplete tests have been described by Fisher (100). To avoid more than two periods and still obtain an estimate of slope, Marks & Pak (195) have used two simultaneous cross-over tests with two different doses (called "assumptions") of the unknown in a so-called "split cross-over test" while Lacey (169) has adopted three simultaneous cross-over tests with three different doses of the sample, giving a still better basis for estimating the log(dose)-response curve.

The experimental error has been reduced in other assays by

testing two or more doses on the same animal. West (264) has assayed the adrenal cortical hormone from the increase of potassium excretion in normal rats on a controlled intake, using two doses of standard and two doses of unknown in a multiple cross-over test. In the assay of coal-tar antipyretics, the segregation of individual differences between cats increased precision to the same extent as a six fold increase in the number of observations while the segregation of differences between dates reduced the error less markedly but yet significantly (42). In their assay of the original and new standards of vitamin B_1 by the rat-curative method, Nelson & Kline (176) obtained the same precision with nineteen rats by eliminating individual differences in overall susceptibility as would be expected with thirty-four rats if each animal had not been used on all three doses.

Using two doses of each of two samples of parathyroid extract in 4×4 Latin squares, Bliss & Rose (35) increased their precision by more than 80 per cent over that obtainable without segregating the differences between individual dogs. The bioassay of digitalis in man (125) has been possible largely because the individual differences in susceptibility have been eliminated. It may be noted that in many other graded-response assays where the same individual can be used twice or oftener (36, 75, 110, 128, 160, 171, 181, 221, 253, 274), it may be possible to increase the precision through the segregation of individual differences in susceptibility.

For an all-or-none response the relative importance of persistent differences in individual susceptibility has been analyzed in detail by Emmens (91) for the mouse assay of the estrogens from the incidence of positive vaginal smears; the results showed the existence of individual levels of sensitivity that were fairly constant relative to each other. A cross-over or similar technique indicated the greater importance of static than dynamic variation in quantal assays of estrone in castrated rats (146). Both Hemmingsen (141) and Marks (194) have applied the cross-over principle to assays of insulin from convulsions in mice, and it is applicable to the assay of intermedin from the expansion of denervated melanophores in *Fundulus* (1).

In assays where the same individual cannot be used repeatedly, variability has been reduced by the use of litter mates. The U.S.P. assay for vitamin A requires that litter mates be distributed but not necessarily balanced between test groups, with an indetermi-

nate effect upon the precision. In an analysis of unpublished data on the vitamin A assay, the variance between litters exceeded that within litters from two to threefold, in contrast with Coward's earlier conclusion (64). Assays of vitamin D in rats, in which litter differences were segregated, showed an increase in precision equivalent to doubling the number of animals whether based upon the bone ash (31) or upon the line test (66). Day-old chicks showed a somewhat higher variability in the percentage of bone ash between than within families (215).

The cross-over and other Latin square designs segregate not only differences between individuals or litters but also between dates or order of treatment. In the rabbit assay of insulin the variability between days has been less than that for individuals but usually larger than the residual error (33, 98). A similar condition has been found in other graded response assays (35, 42) although relatively few as yet have systematically segregated differences due to time or order of treatment.

When different doses of two or more drugs are applied successively to an isolated muscle preparation or animal, a progressive change in susceptibility has sometimes been observed so that in official assays of epinephrine and posterior pituitary extract the standard and unknown are administered alternatively and the entire assay completed in five successive readings (U.S.P. XII). Schild (230) has described a quantitative assay for histamine with the isolated gut of the guinea pig in which two doses of standard and two of unknown were given in a random order in successive replications. The effect of the average change in the height of contraction from one group of four to the next could thus be segregated from the estimate of potency and the error. The policy of adding the higher doses first, as described by Morrell *et al.* (212) in their assay of pituitary extract from a subdivided guinea pig uterus, may bias the slope of the dosage-response curve.

The measurement of slope in a multiple cross-over test has potentially a somewhat lower precision than the comparisons of standard and unknown made on the same individuals. They will have an equal precision where, by means of a larger design than the 2×2 Latin square or its equivalent, two or more doses of either the standard or the unknown or of both are tested on the same individual, but such expansions may be impossible or impracticable. Thus two conflicting objectives arise: (*a*) that of comparing

several treatments—such as two or more preparations and two or more doses of each—on as nearly identical material as possible, and (*b*) the limitation in the number of determinations that can be made upon the same individual and in the brief span when conditions are constant. These requirements are met by the so-called balanced incomplete block designs and their variants (102, 273). The design and calculation of an assay of parathyroid extract with two doses of standard and two doses of unknown but requiring only two treatments on each of twelve dogs has been proposed by Bliss & Rose (35), but Yates (272) has since developed a more efficient method for evaluating such data. These improved techniques have been applied in a simultaneous assay of the relative toxicity of seven organic compounds on aphids (208), testing three compounds in a group.

Correction of quantitative factors.—The variable quantitative factors in biological assays have been handled in several ways. In some cases variations in age, weight, or other conditions have been held within narrow limits by prior selection, and those remaining balanced arbitrarily in assembling groups so that their average initial weights fall within prescribed limits as in the U.S.P. assays for vitamins A and D and for digitalis. A more general type of correction is that inherent in many criteria of response. These are usually empirical but their implied quantitative relations have been tested critically in relatively few cases.

In several assays the value just prior to treatment has served as the base for measuring response. The hypoglycemic reaction to insulin in the rabbit has usually been expressed as a percentage of the initial level with the implicit assumption that for a given dose of insulin the fall in blood sugar was proportional to its initial value. The inadequacy of this premise, first demonstrated by Hemmingsen & Marks (142), has been confirmed by later work (33, 98), which described the correction of the percentage fall in blood sugar from the internal evidence of the assay by covariance. The response to parathyroid extract has been measured as the difference between the initial serum calcium and its level sixteen to eighteen hours later. When assayed with a standard in the same dogs, relative potency could be determined with slightly greater precision from the final serum calcium alone than by the difference from its initial value (35). In the assay of vitamin A from the gain in weight during the test period, Bacharach (10) has shown a

significant correlation between gain and the initial weight in the absence of pre-experimental treatment.

In the adjustment of concomitant measurements, most assays have conformed to an essential rule that the variable for correction should not itself be a function of the dose of drug. Androgens have been assayed from the increase in the weight of the chick comb; Breneman (39) has claimed more consistent results in terms of the ratio of comb weight to body weight but Emmens (91) reported that in his hands the correction for body weight led to no improvement. An unacceptable dosage-reponse equation for the assay of androgens from the weight of the chick comb has been proposed by Frank *et al.* (109) who have disregarded possible variations in susceptibility and slope such as are corrected by comparison with a standard. Their equation is also untenable in that the experimentally determined independent variate (dose) has been solved as a function of both dependent and concomitant measurements (comb weight, body weight, and sex ratio). Assays of known quantitites of androsterone, purportedly to show the value of the equation (162), demonstrate its bias since the equation consistently overestimated small amounts and underestimated large amounts of the hormone. In a study of the variables affecting the assay of gonadotrophic preparations, Gayet *et al.* (119) observed a curvilinear relation between the weight of ovaries and body weight and suggested that corrections might be based upon similar empirical parabolas. Use of the ratio of uterine weight to body weight in this assay introduced an unnecessary error in the experience of Anderson *et al.* (8) since the ratio failed to describe the correct relation.

The use of percentage ash for vitamin D assay in rats implies a quantitative relation between the weight of the ash and the weight of the other constitutents in the dried, fat-extracted bone, which has been questioned. In an experiment by Coward (64) on three doses of standard and three doses of unknown tested on six litters each of six siblings, analysis by covariance showed that in this case potency could be estimated more precisely from the log-weight of ash alone (31). A statistical study (165) based upon the regression of ash-weight upon bone-weight, however, supported the use of per cent ash in the chick assay for vitamin D.

One of the most frequent corrections for a concomitant variable is the adjustment of dose for body weight by a simple ratio. Objective methods have been described for testing the validity of this

assumption (26, 32) and the subject has been reviewed by Clark (60) and by Dawson (71). In an assay of the relative toxicity of sodium arsenite to successive instars of the silkworm larva, the ratio of dose to (body weight)$^{\frac{1}{3}}$ took care of individual differences in size (26); in a digitalis cat assay, the usual ratio of dose to body weight could not be bettered from the available data (32) which was consistent with other studies (132); in a comparison of cardiac drugs in cats where both heart and body weights were recorded, the most effective ratio obtained by covariance was dose to (heart weight)$^{\frac{1}{3}}$ with the body weight then negligible (57). The simpler technique of keeping within moderate size restrictions and giving a constant dose per animal has proved satisfactory in assays where the cross-over principle adjusted differences both in size and in individual susceptibility (169, 194). In other graded response assays where the effect of variations in size is small enough to adjust in the computation by covariance, it is both easier and more accurate to administer the drug at a constant dose per animal rather than per kilogram of animal.

Rejection of aberrant animals or doses.—Aberrant cases may be rejected from evidence taken before or during an experiment or from a study of the data of a completed assay. No bias would be expected from the first procedure which has been used by several workers. In the human assay of digitalis, Gold *et al.* (125) have selected their test cases for steep log (dose)-response curves, rejecting all individuals whose electrocardiograms did not differentiate between three dosage levels differing from one another by 22 per cent. Benson & Edwards (19) have selected pigeons for the emetic assay of a cardiac drug (adonis) by rejecting all individuals that failed to react to 0.065 mg. per kg. of ouabain or did react to 0.030 mg. per kg. The utility of this procedure is limited by possible changes in susceptibility in the month between successive treatments. Swanson *et al.* (249) computed the relation between the gain in weight of 577 rats on vitamin A during the assay period and 6 variables which could be measured by the end of depletion. With this partial regression equation they computed the expected gain for each rat and tried the effect of eliminating all whose expected gains fell outside an arbitrary range of ± 5 gms., amounting to 50 per cent of their animals. The ratio λ computed from their published data show that three rats meeting their requirements would give as much information as four before selec-

tion. Hence the numbers reduced by one half actually gave less information than the full series without selection.

In assays involving operative preparation of the test animal it has occasionally been necessary to hold survivors long enough to insure that hypertrophied glandular remnants have not accounted for a result apparently due to the drug (130). In the assay of cortical extract, Schultzer (232) found that 6.5 per cent of animals alive twenty-one days after adrenalectomy had to be discarded for this reason.

A more controversial matter is the rejection of observations primarily from the magnitude of the results after an assay is completed. The A.O.A.C. assay for vitamin D requires that chicks weighing less than 100 gm. at the end of the assay period be discarded. Loy *et al.* (186) have shown that fewer chicks fail this requirement as the dose of vitamin D is increased so that the chicks heavier than 100 gm. do not represent comparable proportions of the population at the several dosage levels. Since on the standard diet the percentage of ash decreases as the weight increases, this rule gives a biassed result and the authors recommend against rejecting chicks on the basis of body weight. The assumption of an animal standard and failure to recognize the changing susceptibility in a population were presumably responsible for Van Esveld's exclusion of 5 per cent of extreme variates in computing the statistics representative of a long series of digitalis cat assays (258). Heyroth (147) excluded "abnormal" cases in a rat curative assay for vitamin B_1, including individuals which died in the course of the assay or with atypical symptoms but also "resistant rats which remained cured for extremely long periods." Sealey & Sondern (235) considered any response abnormal and omitted it whenever its contribution to the variance of the mean response exceeded the sum of that for all other individuals in the group. This represented less than 1 per cent of their observations. Deletions of the latter type should be based upon statistical criteria such as are reviewed in the next section.

Lack of agreement with the dosage-reponse curve has also served as a basis for rejection. The log-probit relation for quantal assays is presumably linear throughout its length but in occasional series with a clear-cut change in slope at the lower end, the lower doses have been omitted in the computation due to heterogeneity in the stock of animals or a change in type of drug action (25, 29,

73, 115). In most graded response assays, the slope is expected to change as dosages approach either a lower threshold or the maximal reaction. In assays with three or more doses, responses falling outside the linear central part of the curve can be identified by suitable statistical tests and excluded from computations of relative potency (31, 33). Bacharach *et al.* (12) applied this convention in the assay of two preparations of testicular hormones but apparently failed to notice that one response rejected as falling "on the tail of the dosage-reponse curve" was actually intermediate between the two accepted reactions on the other preparation. It is important to consider jointly all dosage-reponse curves in a given assay and reject only on their combined evidence.

Utilization of past experience.—When assays are self-contained so that each provides an estimate of relative potency and its error, evidence accumulates in a given laboratory concerning the slope of the dosage-reponse curve, the standard deviation, the range of responses within which the log(dose)-reponse curve is linear, the regression coefficients for correcting the effect of initial or concomitant measurements, and the conditions which may modify any of these. When combined with the data from occasional tests of procedure, the variation in one or more terms in the assay may fall within the limits expected for random sampling errors. Terms meeting this requirement are said to be in a "state of statistical control" where principles such as those developed for quality control in industry (6, 7) may be practicable. Upon each repetition of an assay, these terms can be compared with past experience by plotting them upon their respective "control charts" or by other more flexible techniques. If consistent, the potency of the particular sample can then be computed with one or more of the values which have been well-established in the past and there is a consequent reduction in the error. If not in agreement, the data can be handled as an independent assay.

The cat assay for digitalis in one laboratory has been studied from this viewpoint by Bliss & Hanson (32), who prepared tables for determining whether the standard deviation in a given assay agrees sufficiently with past experience to justify computing its error with the laboratory standard deviation rather than that estimated from the single test. When the groups are of equal size and number ten or less, the laboratory standard deviation based upon many degrees of freedom can be used alternatively to con-

struct a control chart for the range expected in future groups (218). This chart would indicate whether variation between the animals used in a given test falls within limits expected by chance and provides an objective basis for identifying groups which may include aberrant individuals. Without a knowledge of the expected variation, aberrant observations can be rejected only at a risk of biassing the results.

In other all-or-none assays, the standard deviation is determined indirectly from the slope of the dosage-effect curve in terms of logarithms and probits or their equivalent. The average slope of the dosage-effect curve in frogs for ouabain at the Canadian Laboratory of Hygiene was 21.6 in 1930 (56) and 21.2 in 1939 (4). In eight component curves in 1930, each based upon 240 frogs, the slope varied from 15.3 to 27.8 over a period of three weeks, and due to the large numbers involved, this difference could be established as significant (30). With the smaller numbers used in routine assays, still larger differences in slope fell within the sampling error, from which Allmark & Morrell (4) concluded that the slope of the standard curve could be accepted as stable in their laboratory and used in preference to one determined in each assay. The danger of "borrowing" a curve from another source is illustrated by the significantly steeper slope for ouabain ($b = 21.2$) than for digitalis ($b = 13.1$) which they report and by a number of other studies showing differences in the average slopes for digitalis from one laboratory to another (21, 80, 206). Even though the estimate of slope in a single assay may vary within relatively wide limits by sampling error alone, a succession of such estimates has on occasion revealed a significant shift in the true value. The attainment of statistical control in the past would not warrant the assumption that it will continue in the future as is implied in single-dose assays.

Perhaps the most extensive study on the stability of a graded response has been made in England on the rabbit assay of insulin. Data accumulated in three laboratories in multiple cross-over tests (99) showed a stable average slope for the log (dose)-response curve within two laboratories but over a five year interval a shift toward a steeper slope in a third laboratory. The slope differed significantly between laboratories. Fieller (98) in a more extensive study of part of these data covering a two-year period reported that the slope of the log (dose)-response curve, the standard deviation of percentage fall in blood sugar and the adjustment for varia-

tion in the initial blood sugar level, all varied within the limits expected by chance alone for stable true or "population" values of each term. Given these basic statistical constants, a working estimate of relative potency could be computed from each individual assay by simplified equations, subject to revision if tests failed to confirm the assumption of stability. More limited series of other assays, however, have revealed seasonal variations in slope for the rat growth assay of vitamin B_1 (236) and significant fluctuations from one test to the next as in the assay of vitamin K from prothrombin time (191), of digitalis from reaction time of embryonic chick heart (178), of riboflavin from growth of the rat (89), and of histamine from contraction of the gut of the guinea pig (230). In some of these assays, variations in the standard deviation of response and in the slope of the log (dose)-response curve are correlated sufficiently that λ remains relatively constant.

The sound utilization of past experience in bioassay technique still requires statistics at the professional level, in part because the available experimental data often lack the planned balance which simplifies a calculation and in part because appropriate statistical tools have yet to be described.

TABLE III

TABLE III

Data on Observed Precision of Various Graded-Response Assays

Drug	Animal	Response	Unit of Response	S.D. s	Slope b	λ	Reference
Vitamin A	Depleted rat, female	Gain in weight	5-week period	13.92	49.43	.282 ± .039	Swanson *et al.* (249)
Vitamin A	Depleted rat, female	Gain in weight	8-week period	18.02	88.43	.204 ± .021	Swanson *et al.* (249)
Vitamin A	Depleted rat, female	Gain in weight	4-week period	11.11	22.48	.494 ± .022	Coward (64)
Vitamin A	Depleted rat, female	Gain in weight	5-week period	11.93	27.00	.442 ± .016	Coward (64)
Vitamin A	Depleted rat, male	Gain in weight	4-week period	13.70	38.07	.360 ± .013	Coward (64)
Vitamin A	Depleted rat, male	Gain in weight	5-week period	15.32	45.81	.334 ± .011	Coward (64)
Vitamin B_1	Depleted rat	Gain in weight	1-week period	4.61	19.30	.239 ± .019	Coward (63)
Vitamin B_1	Depleted rat	Gain in weight	2-week period	6.57	38.10	.173 ± .011	Coward (63)
Vitamin B_1	Depleted rat	Gain in weight	3-week period	7.86	54.17	.145 ± .009	Coward (63)
[1]Vitamin B_1	Depleted rat	Gain in weight	2 to 5 weeks	5.0 to 12.3	50.0 to 151.4	.090 ± .005	Various Authors (176)
Vitamin B_1	Depleted rat	Maximum gain on single dose	Grams × days	53.9	928.	.058 ± .016	di Mattei (176)
Vitamin D	Depleted rat	Width of metaphysis	Millimeters	0.186	0.754	.247 ± .023	Schultzer (233)
Vitamin D Standard	Depleted rat	Line test	Degree of healing	0.693	2.013	.344 ± .053	Coward & Kassner (66)
Vitamin D	Depleted rat	Bone ash	Per cent ash	2.948	16.41	.180 ± .018	Shrimpton (239)
Vitamin D	Depleted rat	Bone ash	Log-weight of ash	0.0417	0.596	.070 ± .010	Bliss (31)
[2]Vitamin D	Newborn chick	Bone ash	Per cent ash	3.415	12.64	.270 ± .035	Baird & Barthen (13)
[3]Insulin	Rabbit	Hypoglycaemia	Per cent fall over 5 hours	6.437	45.97	.140 ± .016	Bliss & Marks (33)
[3]Insulin	Rabbit	Hypoglycaemia	Per cent fall	5.863	39.55	.148 ± .011	Fieller (98)
[4]Estrone	Ovariectomized rat	Increase in uterine weight	Weight in mg. per 100 gms.	15.23	132.4	.115 ± .013	Brownlee (41)
[5]Estradiol	Immature rat	Increase in uterine weight	Log-weight of uterus	.049 to .065	.457 to .545	.108 ± .008	Lauson *et al.* (174)
Progesterone	Ovariectomized adult rat	Inhibition of action of constant dose of estrone	Degree of inhibition of uterine estrus	.765	2.988	.256 ± .025	Szarka (250)
[6]Progesterone	Immature rat	Inhibition of action of constant dose of estrone	Degree of inhibition of uterine estrus	.949	3.203	.296 ± .038	Szarka (250)
[7]Pregnant mare serum	Rat	Increase in ovary	Log-weight of ovary	.115	.698	.165 ± .019	Baker (14)
[8]Pregnant mare serum	Immature rat	Increase in ovary	Weight of ovary in milligrams	4.14	34.9	.119 ± .036	Cole & Erway (61)
[9]Gonadotrophic hormone	Immature rat	Increase in ovary	Log-weight of ovary	.193	.603	.319 ± .085	Sealey & Sondern (235)
[9]Gonadotrophic hormone	Immature rat	Increase in seminal vesicles	Log-weight of seminal vesicles	.169	.763	.221 ± .061	Sealey & Sondern (235)
[10]Gonadotrophic hormone	Rat litter mates	Increase in ovary	Log-weight of ovary	.060	.742	.080 ± .007	Bischoff (22)
[11]Gonadotrophic hormone	Immature rat	Increase in uterus	Weight of uterus	14.58	44.28	.329 ± .045	Delís (79)
[12]Insterstitial cell-stimulating hormone	Hypophysectomized male rat	Increase in ventral prostate	Weight of ventral prostate	1.700	7.458	.228 ± .058	Simpson *et al.* (240)
[13]Testicular extract	Rabbit	Dermal bleb	Area in sq. cm.	.0760	5.158	.147 ± .018	Bacharach *et al.* (12)
Chicken pituitary extract	Hypophysectomized lizard	Expansion of melanophore	Degree of color	1.493	3.382	.442 ± .140	Rahn & Drager (221)
Growth hormone of anterior pituitary	Hypophysectomized rat	Gain in weight	Gain in 1 week	4.95	14.0	.354 ± .081	Bülbring (47)

TABLE III (cont.)

DATA ON OBSERVED PRECISION OF VARIOUS GRADED-RESPONSE ASSAYS

Drug	Animal	Response	Unit of Response	S.D. s	Slope b	λ	Reference
Growth hormone of anterior pituitary	Mature hypophysectomized female rat	Gain in weight	Gain in 15 days	6.650	25.12	$.265 \pm .050$	Marx *et al.* (197)
Anterior pituitary extract	Male mouse	Increase in liver fat	Mg. fat per 100 gms. mouse in, 7 hours	79.27	249.6	$.318 \pm .059$	Campbell (52)
Pitressin	Rat	Antidiuretic effect	Decrease in urine over 20 minutes	.272	1.093	$.249 \pm .040$	Jeffers *et al.* (160)
Thyrotrophic hormone	Male guinea pig	Increase in thyroid	Log-weight of thyroid	.0596	.3152	$.189 \pm .024$	Bergman & Turner (20)
[14]Thyrotrophic hormone	Guinea pig	Increase in thyroid	Weight of thyroid in milligrams	12.04	55.47	$.217 \pm .018$	Emmens (93)
Thyroid powder	Tadpole	Decrease in length	Per cent decrease	8.248	21.68	$.381 \pm .107$	Wokes (269)
[15]Parathyroid extract	Dog	Rise in serum calcium	Mg. per 100 cc. after 17 hours	.7265	7.330	$.099 \pm .014$	Miller (200)
Epinephrine	Dog	Blood pressure	Log-mm. Hg	.1277	.633	$.202 \pm .017$	Hjort *et al.* (149)
Ergometrine	Rabbit	Mydriatic effect	Millimeter increase in pupil diameter	.886	6.090	$.146 \pm .037$	de Beer & Tullar (75)
Ergometrine	Rabbit	Hyperthermal effect	°C increase in rectal temperature	.408	2.357	$.173 \pm .038$	de Beer & Tullar (75)
[16]Histamine	Gut of guinea pig	Contraction	Height of contraction			$.034 \pm .004$	Schild (230)
Acetanilide	Cat	Antipyretic effect	Reduction in experimental fever	.651	18.10	$.036 \pm .005$	Brownlee (42)
Urea	Rat	Diuretic effect	Log-increase in excretion	.0396	.7209	$.056 \pm .005$	Lipschitz *et al.* (181)

The "S.D." or standard deviation for a single response has been computed in some cases from the variability within doses, in others from that of the mean responses about the computed curves, occasionally including the differences in slope of replicated series, and in still others from a combination of all sources. In many instances published data did not permit a satisfactory test of the homogeneity of the standard deviation at different doses. The standard deviations reported here, therefore, are the estimates which seemed most pertinent for the precision of the assay in so far as they could be determined from the available data. The slope, b, was computed by least squares for the response as a function of log-dose.

[1] Weighted average of values from eight laboratories in the League of Nations collaborative study, excluding only that reported by von Euler of $\lambda = .026 \pm .004$.

[2] Fifteen eastern laboratories in the AOAC collaborative assay: 5, 10, and 15 unit levels, only, using the official AOAC method.

[3] Per cent fall in blood sugar adjusted for differences in the initial level.

[4] Variation is not independent of dose, hence this value is approximate and limited to fourfold range of dose with the mean weight of uterus 79 mg. per 100 gms.

[5] Variation in log-weight tends to decrease as dose is increased; dosage range from 0.025 to 0.4γ.

[6] Corrected for differences in mean body weight by partial regression.

[7] Variation in log-weight significantly larger at highest doses; dosage range from 4 to 16 "rat units."

[8] Variation in weight of ovary apparently increased markedly as dose was increased; range from 4 to 45 units. Note in these two experiments from the same laboratory that two different functions of ovarian weight are needed to rectify log (dose)-response curve.

[9] Dosage range from 0.75 to 10 international units; standard deviation based entirely on variation of group means about curve, which exceeded approximate value within groups by 2.3 to 2.4 times; latter tends to decrease with increase in dose.

[10] Dosage range 0.075 to 0.30 mg. per animal of prolan; variation in terms of logs is apparently independent of dose.

[11] Author reports dosage-response curve with equation: $y = 98.63 + 44.28 \log (x - 0.27)$, where y is weight of uterus and x is dose of hormone over a sevenfold range.

[12] Standard deviation computed from variability of group means about dosage-response curve.

[13] Slope based upon four similar tests, omitting one low value; standard deviation also includes results of uniformity test.

[14] Valid for expected weight of gland less than 85 mg.

[15] Based on values quoted in reference (35), eliminating differences between dogs.

[16] Value for λ is average of eleven independent determinations; both s and b are highly variable but λ is relatively stable.

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[a] Valid for expected weight or gains.
[b] Based on calculation from Fisher (35), eliminating differences between dogs.
[c] Based on variance of $\log \frac{a}{b}$.
[d] Value for λ is average of eleven independent determinations; both s and δ are highly variable but λ is relatively stable.

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